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Control of Blood Sugar

[A Review Article]

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THE MAINTENANCE of a constant blood-sugar level of around 100 mg. is one of the most important functions of those organs concerned with the proper fuel regulation of the body. These include, besides the obvious 'supply' organs (sensory, central nervous system and digestive tract), most of the organs of internal secretion, and the liver. The co-operative working and interplay of all these tissues to keep the blood sugar at a proper level is one of the most illuminating examples of the constancy of the internal environment of Claude Bernard or homeostasis of Cannon.

There are 3 main factors in this regulation besides the obvious absorption of sugar from the intestinal tract. They are *a*), the rate of oxidation of glucose by the tissues, *b*), the production of dextrose from other substances by the liver, and *c*), the storage of glucose as glycogen, fat, and other derivatives.

The liver has much to do with the adjustments and interrelations of these factors. The organ is to the blood sugar what the heart is to the circulation. The blood-sugar level cannot be maintained without it and most of the other organs that aid in the work, do so by acting through it. The liver, through its functions of storage and release of glycogen, conversion of other substances to glucose, and conversion of glucose to fat, effects the proper adjustments in the blood sugar. When the supply of carbohydrate from the gastro-intestinal tract exceeds the utilization rate of the tissues, the liver largely absorbs the excess and converts it to glycogen and fat. Contrariwise, when the food supply is less than the tissue needs, the liver sets free glycogen and starts converting protein to glucose.

Glucose Needs of Tissues

One of the important endeavors of workers in the field of intermediary metabolism has been the measurement of the rate of glucose utilization, of the tissues of the body. For this purpose one should study the animal in the post-absorptive state in order to eliminate the variables of intestinal absorption and storage of glucose. With these activities eliminated, we have a condition in which the supply of glucose to the blood by the liver closely balances the utilization of it by the tissues.

A requirement for any method purporting to measure this glucose uptake by the tissues is that the animal be maintained in conditions as close to normal as possible. Three methods satisfying this most closely are: *a*), measurement of the glucose requirement of the body after liver removal; *b*), measurement of the sugar output by the liver of the intact animal; *c*), measurement of the glucose utilization of specific tissues or regions of the body in the intact animal. Other methods such as perfusion and tissue-slice metabolism determinations entail having the living agent in such unnatural conditions that it would be

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extremely hazardous to draw conclusions from observations based on them. Tissue culture measurements have interesting possibilities but great caution must be used in drawing conclusions from them.

Measurements in hepatectomized animals. For a period of some hours after surgical removal of the liver from the fasting animal it maintains the normal physiological activity of all other tissues insofar as we can quantify them (1). With the sole source of glucose removed, the sugar in the blood will steadily drop as the tissues use it up. The utilization rate can then be measured by determining the rate at which glucose must be injected to maintain it at a constant level in the blood. Mann (2) reports that this rate is 250 mg. per kg. per hour in the dog; it is 125 mg. per kg. per hour in the rabbit (3). Russell (4) reports the utilization by the eviscerated rat as 135 mg. per kg. per hour.

Output of sugar by the liver. Undoubtedly the method of choice for measuring the glucose utilization of the body would be the determination of the ouput of this substance by the liver in the intact animal. The concentration of sugar in the blood entering and leaving the liver can be accurately estimated. In actual practice, however, this method has many pitfalls. It is extremely difficult to obtain true blood samples without disturbing the circulation of this organ. Furthermore the blood flow through the organ must be very variable, or the estimation of it must be precarious, in order to give the wide range of figures that have been reported for its value (5–10). Since workers who have measured blood-sugar differences between blood entering and leaving this organ have used average figures for blood flow of other observers their results contain the error[1] inherent in taking the product of the averages of two series.

Wierzuchowski and Fiszel (11), on anesthetized operated dogs that had been fasted 28 hours, found an increase of 15.6 mg. of glucose per 100 cc. of blood in its passage through the liver. They estimate this as indicating that the liver supplies the tissues 228 mg. of glucose per kg. per hour. Heller (12) found an increase of 17.2 mg. per 100 cc. of blood going through the liver which he calculates as equivalent to a new production of glucose of 240 mg. per kg. of body weight per hour. The blood sugar and blood lactic-acid levels of his animals are surprisingly high for fasted phlorizinized animals and would indicate a considerable divergence from normal conditions caused by the operative procedures and anesthetic. Cherry and Crandall (13) made use of the London cannula technique in unanesthetized dogs and, judging from their description and the blood sugar and lactic acid levels, the conditions in their experiments must have been about as close to natural as is possible. They did not measure blood flow through the liver but using the average figure of Blalock and Mason (14), they calculated that the liver supplies sugar to the blood at the rate of 150 mg. per kg per hour in dogs fasted 18 hours. Recently, Crandall and Lipscomb (15) have developed a method for simultaneous determination of blood flow and blood-sugar difference for the liver. They determine the blood flow by determining the urea difference in the blood entering and leaving the liver and dividing this difference into the amount of urea excreted in the urine in unit time. They report that the glucose production never exceeds a rate of 160 mg. per kg. per hour.

Measurement of individual tissue utilization. The determination of the drop in blood sugar on passing through the other tissues of the body can also give valuable information concerning glucose utilization. It should be carried out on the intact unanesthetized animal and great care must be observed in the taking of the blood samples lest effects be produced on the blood flow or the tissue metabolism. The blood-sugar difference should be corrected for lactic-acid increase. The determination of the glucose utilization of the whole body with this method would be tedious, requiring arterio-venous blood-sugar differences and the blood flow determinations for all regions of the body.

In dogs fasted 18 hours Cherry and Crandall (13) found the arterio-venous blood-sugar difference for the legs to be 4.8 mg. per cent and for the intestines 2.9 mg. per cent. The simultaneous increases in blood lactic acid were 3.1 and 0.68 mg. per cent, respectively. For the leg of the 18-hour fasted dog Cori, Fisher, and Cori (16) report an average of 2.2 mg. per cent blood-sugar decrease for 13 determinations, and an increase of 1 mg. per cent of blood lactic acid in 2 determinations. Earlier work does not differ essentially from that reported by these two groups, and is reviewed by them. Wierzuchowski and Fiszel (11) determined the arterio-venous blood sugar and blood-lactic acid differences on 11 dogs fasted 8 hours. The average decreases in sugar of blood passing through muscle, head, and intestines were 4.6, 10.3, and 1.9 mg. per cent, respectively, and of these, blood lactic acid increases accounted for 56, 14, and 164 per cent, respectively.

[1] This error may be illustrated with the following simplified case.

Suppose the output of sugar by a given liver is constant but the blood flow through it is variable.

	Bloodflow per minute	A-V difference	Output of sugar per minute
Condition (1)	100 cc.	1 mg.	100 mg.
Condition (2)	10 cc.	10 mg.	100 mg.
Averages	55 cc.	5.5 mg.	302.5 mg.

The interesting feature of this error is that its always gives values higher than the actual values. There can be no hope of minus and plus errors cancelling each other.

Effect of Fasting on Sugar Utilization of Tissues

Hofmeister (17) many years ago reported that the fasting organism is more susceptible to alimentary glycosuria than the well-fed one. This condition, which has been described by many others since, is known as 'starvation diabetes.' This might suggest that the tissues of the fasting animal use less sugar than those of the fed, but not necessarily so. The blood-sugar curve after feeding sugar does not give us any information about tissue utilization prior to the feeding. It would give some indication of this for the period after feeding but this will depend on mechanisms such as hormones, put into action as a result of the feeding. To determine this we must keep the blood sugar at its normal level and avoid the action of the liver in storing sugar or in making new sugar. This means using one of the special techniques mentioned previously.

Bergman and Drury (18) have shown that a preoperative fast diminishes the sugar requirement of the eviscerated rabbit from 206 mg. per kg. per hour for fed animals to 110 mg. per kg. per hour for those fasted 4 days. No corresponding study of dogs has been made, but Mirsky (19) reports 140 mg. per kg. per hour maintains the blood sugar of the 4-day fasted dog after evisceration, which is lower than the figures given by other workers for unfasted dogs. Drury, Bergman, and Greeley (20) found that 80 mg. per kg. per hour maintains the blood sugar of the hepatectomized dog that has been fasted and phlorizinized for 4 days before operation. Crandall et al. (21) using, the London cannula technique, have found that fasting for more than 3 days reduces the amount of glucose liberated by the liver into the hepatic vein almost 50 per cent.

These results are in keeping with the findings with tissue and urine analyses during the first day of the fast. During this period the liver glycogen drops rapidly indicating a considerable supply of glucose from this source. In the dog it would appear that glycogen continues to be given up in considerable quantities in the second and even third days. Assuming the liver as 2.7 per cent of the body weight and containing 10 per cent glycogen at the start of the fast this could readily supply 100 mg. of glucose per kg. per hour for 24 hours and still have an appreciable amount of glycogen left. Benedict (22) estimates 65 and 25 gm. of glycogen, respectively, metabolized during the first and second days of fasting in man.

Not only is there evidence of a supply of glucose from glycogen during the first day or two of a fast, but there would seem to be more protein metabolized per hour as compared with later days of fasting. The evidence for this is summarized by Lusk (23) and shows this extra protein metabolized in the first days of a fast may give rise to as much glucose as that coming from glycogen. There is, then, during the first two or three days of fasting, utilization of both liver glycogen and protein. Later in the fast, protein becomes the only source of glucose and this utilization is diminished in amount.

The evidence from these divergent sources indicates that the tissues of the dog are supplied with between 200 and 300 mg. of sugar per kg. per hour during the first day of fast and that by the fourth day it has been diminished to between 50 and 100 mg. per kg. per hour. The time required to bring about this change does not necessarily obtain for other species; by and large the smaller the animal, the more rapid the change. We might reasonably expect the mouse to show as much change in 24 hours as does the dog in 4 days.

This indicates that during extended fasting, but a small fraction of the total fuel requirement of the tissues is supplied by glucose, perhaps not more than 10 per cent. In view of the fact that the fuel of the central nervous system seems to be almost entirely glucose (24), one might well ask if the requirement of this tissue does not account for all of this substance used by the fasting organism. Mulder and Crandall (25) find the arterio-venous difference for the brain to be just as great (10 mg. of sugar per 100 cc. of blood) for the 6-day fasted dog as that of the recently fed animal. In the present state of our knowledge of metabolism it would be best to recognize two types of glucose utilization by tissues, one peculiar to the brain and the other shared by all other tissues. The rate of the former is high and practically constant under all conditions. The rate of the latter varies greatly according to circumstances. It is high in normal, fed animals, and is low in fasting ones.

Production of Glucose from Other Substances by the Liver

Conversion of protein to glucose. The work of Bollman and Mann (26) definitely shows that this transformation is carried out only by the liver. Much work has been done attempting to determine how much glucose can be obtained from a given amount of protein. Earlier workers believed that the amount of glucose obtainable was 40 to 60 per cent of the weight of protein transformed. These values were based on results obtained in depancreatized and in phlorizinized dogs. When these animals were fed protein, extra sugar was excreted in the urine in an amount representing 40 to 60 per cent of the fed protein. The assumption was made that the tissues of these animals used no glucose. In the light of recent work which shows that at least one important tissue, the central nervous system, uses glucose almost ex-

clusively at all times and under all conditions (24), this assumption can no longer be maintained. Drury Bergman, and Greeley (20) have measured this glucose utilization by the tissues of the phlorizinized dog. When this amount is added to that excreted in urine, a total is obtained which would necessitate the conversion to sugar of 80 to 90 per cent of the catabolized protein in order to supply this. Results in protein feeding experiments with depancreatized dogs confirm this conversion figure (27).

At the beginning of a fast the glucose needs of the body come from stored glycogen and protein which is converted by the liver. The glycogen stores in small animals such as the rat are largely exhausted in a day or two; they last as long as 3 or 4 days in larger animals such as the dog and man. However, as the fast continues the body depends more and more on protein for its sugar. After 4 days of fasting this is probably the sole source. During the first 2 days of a fast this protein comes largely from that stored by the liver itself. After this the other tissues yield relatively more protein (28). After the third and fourth day of fasting the glucose needs of the body are supplied almost entirely by the liver from conversion of protein from the general body tissues.

Little is known concerning the mechanism controlling the breakdown of body protein under physiological conditions. Under ordinary circumstances the controlling mechanisms adjust this breakdown nicely to the glucose utilization of the tissues so that we do not get large imbalances with corresponding fluctuations in blood sugar. Recent work (29) has shown that it is not exactly correct to speak of body protein breakdown and of body protein regeneration as two distinct and mutually exclusive processes. These two processes are going on simultaneously at all times, it is the balance between them that shifts. Thus when the body is in 'negative nitrogen balance' the process of body protein breakdown is greater than that of regeneration. The processes of breakdown and regeneration of body proteins are not simply the reverse of each other, physiologically speaking. Body protein breakdown means setting free amino acids which go to the liver to be changed to glucose together with urea which is excreted. Body protein can give rise to glucose in this way, but we cannot make body protein by feeding glucose, although we can diminish the breakdown during fasting by giving glucose. The liver is an important factor in this control of breakdown and building of body protein. It converts the amino acids from the breakdown to a ready fuel, glucose, and when the body is building up protein it must allow the amino acids of the food to pass through it and not change them to glucose. In severe diabetes we have a state in which the liver is not properly controlled in this respect; the liver converts to glucose any exogenous protein which thereby is useless to the tissues for building up purposes even though they may be greatly exhausted in protein material. The mechanism of the control of this activity of the liver remains one of the most important problems of protein metabolism yet to be worked out.

The mechanism of the control of body protein breakdown is also a problem needing investigation. It seems to be closely related to the glucose utilization of the tissues. When the latter is high, e.g. after carbohydrate feeding or insulin administration, the rate of body protein breakdown is very low. When it becomes low, as in fasting, there is an increase in body protein breakdown. In untreated diabetes there seems to be a low rate of tissue utilization of glucose during fasting and a high body protein breakdown is to be expected. One can simplify our ideas about body protein breakdown and carbohydrate metabolism if one takes the concept as a working hypothesis that any condition which decreases glucose utilization by the tissues increases body protein breakdown. Conversely, when we have a condition in which glucose utilization is low and body protein catabolism is high, we will find that this latter will be decreased by any mechanism which increases the former. By utilization one does not necessarily understand oxidation of glucose since transformation to intermediate forms may serve as well.[2] At the present time very little is known about the immediate fate of glucose which disappears from the body fluids. A clear distinction must be kept between blood-sugar level and glucose utilization rate. As will be illustrated later in the paper, the blood-sugar level may be high with a simultaneously low utilization rate, or vice versa. This point will be reviewed later after having taken up special cases illustrating it.

Conversion of other substances to glucose. The liver can convert many other substances such as lactic acid, pyruvic acid, and other monosaccharides into glucose. The possibility of conversion of fat to sugar by the liver is always a very tempting idea. In taking recourse to it the theorist saves himself a great deal of bother with exact study of nitrogen and glucose balances. However, this concept has not been the theory of those who have insisted on rigid observance of extreme care in experimental procedures and care-

[2] By utilization is meant the change of glucose to some other chemical state which can be disposed of, or deposited in a form in which it can remain indefinitely without prejudice to the body's normal functioning. Complete oxidation is such a process since the chemical products, CO_2 and H_2O can be readily disposed of. Conversions to glycogen and fat are also such processes since the products can be deposited and stored without interference to the normal economy of the body. Conversion to lactic acid or pyruvic acid could not be so classified since these substances cannot be deposited or readily excreted. Rather, they accumulate in the blood and body fluids and will, and if this is persisted in, interfere with the normal physiological operation of the body.

ful analysis of results. In the past, many claims of a demonstration have been made but all have fallen down on subsequent checking of the experiments. However, at the present time, we cannot claim the impossibility of conversion of fat to sugar. It is possible that some day a definite demonstration of this transformation may be forthcoming. Until such time it is better to discard the idea not only because it has not been proven, but because it is unnecessary. The facts of metabolism and diabetes can be more simply explained and understood without this complicating hypothesis. Besides this, there are some suggestive facts which point against it. Thus, the feeding of fat to the diabetic or phlorizinized animal does not lead to an increased excretion of sugar as does protein feeding. This, its proponents say, is because the liver is already handling all the fat it can. However, there is evidence of the liver doing something to the extra fat, for an increased ketone body production occurs. Furthermore, such a liver will produce sugar if supplied with fatty acids containing an odd number of carbon atoms (30).

Conversion of glucose to storage products by the liver. The capacity of the liver to store glucose in the form of glycogen is well known. This organ can store glycogen up to a concentration of 10 per cent. This accounts for more glucose than it might seem at first glance, since the weight of the liver will be as much as doubled when it lays down such large amounts of glycogen. Water seems to account for most of this extra weight. The liver is ordinarily considered as an important factor for the conversion of glucose to fat. However, it has been shown recently that under certain conditions rats with the abdominal viscera removed can have a respiratory quotient above 1.1 when they are given glucose (31). This work indicates that both the liver and peripheral tissues can be sites of fat formation from carbohydrate. This process will be taken up later in the sections on the different endocrines.

GLANDS OF INTERNAL SECRETION

The three important factors affecting the blood sugar, *a*), tissue utilization, *b*) storage, and *c*), new formation of glucose are regulated in a remarkably efficient manner since ordinarily little change in blood-sugar level occurs despite huge variations in carbohydrate intake and metabolic expenditure. There is no evidence that the peripheral tissues can of themselves vary their glucose consumption to the desired rate needed for the varying conditions of nutrition and metabolism. Thus, any given cell in the periphery does not know that the animal is eating *a*), a high carbohydrate diet at one time and hence should increase the proportion of glucose in its fuel, or *b*), know that the animal is fasting and hence should lower glucose utilization and increase that of fat. The concentration of glucose in the blood may have some small direct effect on the rate of utilization by the body cells (32). The changes in concentration needed to produce such effects are quite outside the physiological range in which the normal body manages to keep, despite large fluctuations in intake and fuel expenditure. This regulation of the type of fuel mixture that the tissues use seems to be effected by the endocrine system. The endocrines also regulate the rate of conversion of glucose to storage products. Their rôle in the new formation of glucose from body protein is uncertain.

The methods of study of these organs are common to all of them. One procedure involves surgical removal of the organ followed by study of the resulting changes in metabolism. The other method consists of studying the changes in metabolism resulting from administration of the active principles of the organs in normal subjects or into those previously deprived of the organ in question. Nature, by disease, sometimes produces the experimental conditions in man which the experimentalist produces in animals by the aforementioned methods.

Adrenalin

The immediate rise in blood sugar produced by adrenalin is the result of breakdown of liver glycogen to form glucose. This hormone also causes muscle glycogen to break down to lactic acid. The latter is carried by the blood stream to the liver where it is changed to glucose or glycogen. Adrenalin, then, will cause a primary drop in liver glycogen followed by a rise, which in some species, may carry it to higher levels than those of control animals. Adrenalin seems to be an agent which liberates stored sugar (glycogen) into the blood for extra needs in emergency conditions.

Thyroxin

Thyroxin does not seem to have any immediate effect on carbohydrate metabolism. Continued administration of this hormone results in depletion of glycogen stores. At this stage the animal may become hypoglycemic, be hypersensitive to insulin, and show relatively little response to adrenalin. The changes do not necessarily indicate any specific action of thyroxin on carbohydrate metabolism. These changes may mean merely carbohydrate depletion resulting from the generally increased metabolic rate. As a result of the latter, all stored foodstuffs will tend to be depleted.

Insulin

Mode of action of insulin. The action of insulin at the present time cannot be completely explained as one process; there are at least three actions related to

carbohydrate metabolism which may be produced under different conditions.[3] Whether these three are all resultants of one fundamental underlying process cannot be answered at this time and with our present knowledge of metabolism. These three actions are: *a*). Inhibition of excessive breakdown of body protein to form sugar. *b*). Increase in storage or disposal of fed carbohydrate: that is, assimilated carbohydrate that is not needed for immediate current needs but which is put away for fuel needs at a later time. The known forms in which this can be disposed of are muscle and liver glycogen, and body and liver fat. *c*). Increase in immediate oxidation of glucose.

These effects have been demonstrated in the depancreatized dog and cat. These diabetic animals when not given insulin, exhibit *a*) excessive breakdown of body protein, *b*) an inability to store fed carbohydrate and *c*), some impairment in capacity to oxidize glucose. Administration of insulin to such animals corrects these deficiencies and so brings about the actions listed above.

It is only in recent years that the effects of pancreatectomy in such animals as the goat, monkey, and rabbit have been studied (33–37). The resulting diabetes is much milder than in the pancreatectomized dog and cat, so that the animals can survive without insulin administration. The animals can maintain their weight and keep in nitrogen balance without severe ketosis on a high carbohydrate diet. Insulin seems necessary to produce an increase in weight on such a regime (38). The results in these animals show that insulin is not a necessity for the oxidation of glucose. Furthermore, the capacity of the tissues of these animals to utilize appreciably large amounts of carbohydrate is probably the reason they can be maintained in nitrogen balance; there is not the stimulus to tissue protein catabolism which, in the depancreatized dog, is the low rate of glucose utilization of the tissues. The results in these animals should be given more attention in forming our ideas of insulin action. Our notions in this connection have become too fixed by findings in the depancreatized dog. That this animal was the first to be depancreatized and was the only preparation studied during the early years of investigation in this field, is no reason for its tyrannizing our thinking in this regard. The depancreatized goat and monkey, although studied more recently, should have just as much weight in our consideration and conclusions. The depancreatized rat can be maintained in good condition on a high carbohydrate diet without insulin. He can keep in nitrogen balance, ketosis is not present and he utilizes a considerable amount of sugar. On such a diet he cannot gain weight without insulin, and any weight lost during a fast cannot be regained by subsequent feeding except with the aid of insulin (38). On a diet containing appreciable amounts of fat he will readily put on weight.

Recently Tepperman et al. (31) have found that rats can be trained to eat their daily food in 3 hours. When such rats are eviscerated and given insulin and excess glucose they show respiratory quotients of 1.1 or over, indicating that the peripheral tissues of these trained animals can convert glucose to fat. Untrained control rats show respiratory quotients of less than 1 when similarly treated. These results suggest that some long-acting hormone cooperates with the insulin to make possible the conversion by the peripheral tissues of the trained rats. Since, in the untrained intact rats, fat synthesis can occur it would seem that the liver is the important site for the conversion in them and when this organ is removed this process is largely stopped even when insulin and glucose are given.

It is generally assumed that insulin increases the immediate oxidation of glucose although the proof of this is not as simple as was once thought. Writers at first believed that insulin had this action because at that time the prevailing theory maintained that the trouble in diabetes was due to an inability to burn glucose. Since the use of insulin was successful in the treatment of this disease, it was assumed it must have increased the oxidation of this sugar. More recent work has furnished good evidence that the depancreatized animal can oxidize glucose in not inconsiderable amounts. This follows from the observation that when the liver is removed from the diabetic animal, the blood sugar falls and glucose must be given in order to keep the animal from going into hypoglycemia. Yater et al. (39) found that the rate of glucose injection necessary to maintain a constant blood-sugar level in a depancreatized-hepatectomized dog was 160 mg. per kg. per hour in contrast to a rate of 250 mg. per kg. per hour needed for the hepatectomized dog. Greeley and Drury (40) find that fasted diabetic rabbits after evisceration need as much glucose as normal fasted rabbits after hepatectomy. The glucose needed for the diabetic dog may be largely that needed for the brain, and the results may indicate some impairment of utilization by other tissues. In the rabbit and other herbivora utilization by both the brain and the other tissues of the diabetic animal is probably normal for basal conditions, but is less than normal in the fed condition. The impairment of glucose utilization in the diabetic is, then, not absolute, but varies with the tissue, the species, and the

[3] These are the important general effects of insulin ordinarily considered in clinical and metabolic work. Other specific actions have been reported such as increase in pyruvic acid formation (50) and increase in phosphorylation of glucose (51). These would seem to be related to carbohydrate metabolism and may be intermediate steps or reactions secondary to the general effects stated above.

physiological state of the animal. Insulin certainly increases this utilization but to what extent by increasing storage of glucose and to what extent by increasing immediate oxidation is not easy to determine. Some workers have attempted to measure the extent of this latter action by determining the rise in R.Q. after insulin. However, although a rise in R.Q. might be due to an increase in immediate oxidation of sugar, it could as readily result from an intermediary change of glucose to some other compound containing less oxygen.

The chief effect of removal of the pancreas, then, is a resultant diminution in utilization of glucose by the animal, particularly for storage. There are significant quantitative differences in the various species but these differences apply only to tissues other than the brain. The utilization of glucose by the brain remains high in all species after pancreatectomy and accounts for a definite portion of the glucose requirement of all depancreatized animals after hepatectomy. The utilization of the other tissues in diabetics varies with the species. In the rat the utilization is sufficient for current needs but is not adequate for storage.

Can it be that insulin has but one pharmacodyamic action and its apparent multiple effects are the results of this fundamental action reacting on different physiological mechanisms? Insulin accelerates the disappearance of glucose in the body. The glucose might well pass through some first chemical stage which could be a bottleneck for the different routes which carry glucose to its different fates, oxidation, or storage as fat, or glycogen. The concentration of the compound representing this first chemical stage might affect secondarily the rate of protein catabolism by the tissues in general. Insulin is not absolutely necessary for the maintenance of nitrogen balance. Most depancreatized herbivora stay in nitrogen balance when fed without insulin. More than a normal amount of body protein is not broken down when they are fasted.

Pituitary

Hypophysectomized animals can survive the operation for long periods and in good condition, provided they are fed adequate amounts of carbohydrate or protein. They show a marked drop in blood sugar on fasting, which, in the rabbit usually leads to a fatal outcome in a few hours (41). The fasting hypoglycemia is serious in the rat and often so in the dog. The blood sugar of the hypophysectomized rabbit can be kept at normal levels by constant injection of glucose; Greeley (41) has found that these animals need almost 600 mg. per kg. per hour to prevent hypoglycemia. In the same way, Russell (4), determined the sugar need of eviscerated rats and eviscerated hypophysectomized rats. For the former, the rate is 135 mg. per kg. per hour and for the latter 250 mg. per kg. per hour.

The hypoglycemia of the fasting hypophysectomized animal has been explained by many authors (42) on the supposition that these preparations are greatly restricted in their capacity to break down body protein for the formation of new glucose. Such a hypothesis cannot account for the differences observed by Russell as noted above, since all of these animals were eviscerated and hence in all, new formation of glucose would be stopped. Even less is this idea capable of accounting for the findings in the rabbit. The normal fasting rabbit breaks down body nitrogen at the rate of 20 mg. per kg. per hour. This would give rise to about 120 mg. of glucose per kg. per hour and this is enough to balance the sugar needs of the normal animal. Therefore, even if all protein catabolism were stopped in the hypophysectomized rabbit (as after hepatectomy) the sugar need would increase only to this figure. Actually it is close to five times this amount after hypophysectomy.

The hypophysectomized dog metabolizes protein of the food to the same extent as the normal one. Fasting reduces its nitrogen excretion about 25 per cent below that of the normal. This indicates a reduction in breakdown of tissue protein. However, the percentage reduction of this metabolic process is just about the same as that for the general reduction in basal metabolism. When both types of animal are given phloridzin, in addition to fasting, the difference in nitrogen excretion becomes much greater. The difference in all metabolic processes is probably greatly increased since the blood sugar of the hypophysectomized animals sinks to very low levels under these conditions and they would probably have low body temperatures. Many of them are morbid and some die. The lower nitrogen excretion is probably only partly due to this general slowing up in the metabolic processes. If the rate of utilization of glucose by the tissues is increased by the hypophysectomy there would not be the stimulation for increased body protein breakdown.[4] Some of the results of Houssay and Biassoti (42) show this increase in sugar utilization by the dogs deprived of the pituitary. Table 1 shows the effect of fasting and phloridzin on

[4] At first sight the results of Lee and Ayres (52) might appear to contradict this view. They fed normal and hypophysectomized rats exactly the same amount of food over a period of 30 days. At the end of this time it was found that the hypophysectomized rats had lost more body protein and less body fat than the controls. This may indicate an increased body protein breakdown. However, it could result from a reduced regeneration rate which would be secondary to an increased conversion of food protein to sugar by the liver. In other words, the liver might convert practically all food protein into glucose and the tissues of the animal would be in a condition similar to those of an animal eating plenty of carbohydrate but insufficient protein, that is, it would be in negative nitrogen balance.

TABLE 1

	(1)	(2)	(3)	(4)
	Glucose	Nitrogen	N×5.50	Glucose Util. (3 minus 1)
Hypophysectomized	0.64	0.40	2.20	1.80
Controls	2.34	0.67	3.69	1.26

the glucose and nitrogen excretions of hypophysectomized and of normal dogs, both fasting.

Column 3 gives the glucose produced from the body protein breakdown assuming that each gram of protein nitrogen is equivalent to 5.5 gm. of glucose. In *column* 4 is given the glucose utilization by the tissues, i.e., the glucose produced (*column* 3) minus that which was excreted (*column* 1). Not all series show this difference but this is cited to show that glucose utilization may, at times, actually be higher in the hypophysectomized animals.

The study of anterior pituitary function by injections of extracts of the gland is complicated by the possible effects of the extracts on the adrenals and thyroid through the adrenotropic and thyrotropic hormones. To some extent, then, effects on carbohydrate metabolism may be secondary to the action on these glands. However, there is clear evidence that extracts of the pituitary act on carbohydrate metabolism without the intermediation of other ductless glands (43).

There seem to be two factors in the anterior pituitary which affect carbohydrate metabolism, one exerting its action soon after its injection and for a period of a day or two thereafter; the other producing an effect which persists indefinitely. The action of the former is brought about by injection of the whole gland extract and is evidenced by the rise of blood sugar of normal animals, the increase in glycosuria of partially depancreatized animals, and the counteracting of the huge glucose need of hypophysectomized rabbits. The factor acting for a longer period of time was discovered by Young (44, 45) who found that dogs given daily injections of crude pituitary extract of absolutely fresh glands for a week or so became permanently diabetic. These animals developed degenerative changes in the pancreatic islet tissue (46) and low insulin content of the pancreas.

Adrenal Cortex

In many respects the adrenal cortex acts on carbohydrate metabolism in a manner similar to that of the anterior pituitary. Adrenalectomy in fasting animals leads to depletion of the liver and muscle glycogen and to hypoglycemia (43) and there is a lower protein catabolism than in normal animals. Administration of cortical extract to normal or adrenalectomized mice and rats causes an increase in liver glycogen and a rise in blood sugar (43). Long and Lukens (47) showed that adrenalectomy attenuates the diabetes of the depancreatized cat in a manner similar to hypophysectomy. In partially depancreatized rats adrenalectomy decreases the glycosuria and cortical extract injections increase it. The general condition of fasted hypophysectomized rats is improved by injection of cortical extract and at the same time they show an increase in the liver glycogen and blood glucose. The urine nitrogen is also increased. These findings might suggest that much of the effect of the anterior hypophysis on carbohydrate metabolism may be mediated through its adrenotropic action. However, anterior pituitary extract has a diabetogenic action (increased blood sugar and glycosuria) on adrenalectomized depancreatized animals (43) which shows that the extract can act directly without any secondary action by the adrenals.

Long et al. (43) find that nitrogen catabolism in rats is reduced after adrenalectomy and is increased in such animals by cortical extract. They conclude from this that one of the properties of the cortical hormone is a stimulation of protein catabolism and that the increased blood-sugar levels following its injection into animals is an expression of this effect. However, we may consider this point in the same way as in discussing the anterior pituitary. The increased protein catabolism may be the result of the decreased sugar utilization by the tissues, which results from the action of the cortical hormone. We have again the question of whether removal of the adrenals results in lower body protein conversion to glucose with subsequent hypoglycemia, or whether adrenalectomy brings about increased glucose utilization by the tissues and this decreases the breakdown of body protein and hence there is less new glucose formation.

Some workers have attempted to determine the protein catabolism of adrenalectomized animals by subjecting them to fasting and phloridzin and thereby straining the protein catabolism (48). They report that such animals break down less body protein than normal fasting phloridzinized animals. It is inferred that the loss of the adrenals interferes with the breakdown of body protein. There appears to be a more probable cause of reduced protein catabolism in these animals. These animals have all metabolic processes retarded, evidently because of very low blood sugar. The temperature is reduced (43). The oxygen consumption is low (49). Indeed many animals are near death. Why should one assume that the metabolic process of protein catabolism does not behave like other metabolic processes and be reduced? That it is the low blood-sugar level that retards metabolism is indicated from the results obtained on giving these

rats sugar. Oxygen consumption increases and the temperature rises. The giving of sugar actually increases the breakdown of body protein (48).

Additional support of the view that the increased protein metabolism is secondary to increased sugar utilization is found in the results of Long and Lukens obtained in depancreatized adrenalectomized cats. The following figures give the nitrogen excretion of their animals as given in table 2 of their paper.

Experimental Condition	Fasting Nitrogen Excretion
Normal, fasting	0.6
Depancreatized	1.4
Adrenalectomized and depancreatized	0.6

It is apparent that the adrenalectomy reduces the high protein catabolism of pancreatic diabetes to a normal level. Despite this normal protein breakdown most of their animals show hypoglycemia. In table 4 of their paper they report the blood sugars of 11 of their adrenalectomized-depancreatized cats after an overnight fast. Of the 11, 7 were markedly hypoglycemic, 2 had normal blood sugar values and 2 were hyperglycemic. The same animals were not necessarily reported in the two tables but the results are suggestive. If the hypoglycemic animals reported in table 4 had normal or slightly subnormal protein breakdown (which was the case for those reported in table 2), these animals must have had glucose utilization rates higher than normal since their blood sugars went to levels so much lower than that of normal fasting cats.

If, then, these animals have rates of glucose utilization that are normal, or higher, the lower results for nitrogen excretion are to be expected since we have removed the incentive for increased nitrogen catabolism.

Endocrines and the Protein-Carbohydrate Relationship

We may sum up here the bearing that the endocrines have on carbohydrate and nitrogen metabolism. In the carnivora, removal of the pancreas results in a decrease in carbohydrate utilization and in an increase in body protein catabolism during fasting. These effects are reversed by supplying insulin. In herbivora, pancreatectomy brings out an inability to store carbohydrate but does not reduce to any marked extent the capacity to utilize glucose for current needs and does not bring about any increase in body protein catabolism during fasting. The removal of the pituitary increases the utilization of glucose and decreases a supernormal body protein breakdown when this is present. This effect is particularly evident in diabetes. The removal of the adrenal glands brings about results similar to those caused by hypophysectomy. Injection of the extracts of these glands usually produces effects opposite to those of removal of them.

If we accept the concept that increased utilization of glucose by the tissues results in lowering of the protein catabolism of these tissues, the known facts of endocrine function in intermediary metabolism can be greatly simplified. We need not suppose a distinct and separate protein action for each of the endocrines acting on carbohydrate metabolism. If this latter action operates, then the effect on protein metabolism would result from a physiological process common to all these situations. It is apparent that the blood-sugar level of itself cannot be the factor determining tissue protein breakdown. The blood sugar is high in depancreatized carnivora and there is a large breakdown of body protein. This latter can be brought to normal either by hypophysectomy or adrenalectomy which on the other hand occasion a very variable blood sugar level. The blood sugar is very low as a result of fasting and phloridzin and here there results a high tissue protein breakdown.

Summary of Endocrine Relationships

It is apparent that the endocrines play a very important rôle in regulating the mechanisms affecting the blood-sugar level and concerned with maintaining it at a proper level. Adrenalin releases glycogen when glucose is needed quickly. Insulin increases utilization of glucose particularly by storage routes, and restrains an excessive new formation of glucose from protein. The anterior pituitary and adrenal cortex inhibit the utilization of glucose by the tissues. The action of these two latter glands, under normal conditions restrains the otherwise large consumption of sugar by the body. When they are removed, the tissues, freed from their curbing action, consume greatly increased amounts of glucose. It seems, then, that the average body cell, freed of all hormone constraint uses a large amount of glucose. Evidence for this is seen in the high rate of sugar utilization of the hypophysectomized eviscerated animal (41) and of the ordinary cell grown in tissue culture (32). In the physiologically functioning subject this is ordinarily reduced by pituitary and cortical action. The insulin mechanism increases glucose utilization on occasions of large carbohydrate intake.

SUMMARY

Despite marked variation in feeding, the blood sugar is kept at a normal level by the proper regulation of tissue utilization, conversion of glucose to storage products, and new formation of glucose by the liver. These mechanisms are largely controlled by the endocrine system. When carbohydrate intake is high, glucose is stored as glycogen and fat as a result of insulin activity. In the post-absorptive state insulin activity decreases, thereby stopping storage, later allowing glycogen to be released and still later result-

ing in breakdown of body protein with resultant new formation of glucose. Glucose utilization by the tissues is increased during feeding (200–250 mg. per kg. per hour) and this is gradually cut down by fasting to 100 mg., or less, per kg. per hour. This control is independent of insulin and might well be effected by the anterior pituitary and adrenal cortex.

The living cells of the body, separated from all hormone activity, seem to have a very high rate of glucose utilization. In the body under physiological conditions, this rate is markedly curbed by the functioning of the pituitary and adrenal glands.

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'Spontaneous' Variability of Oral Glucose Tolerance

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DURING THE COURSE of an investigation of the carbohydrate metabolism of non-diabetic subjects we have been struck by the frequent occurrence of wide variations in the blood-sugar levels of individuals on repetition of the oral glucose tolerance test. Since this procedure is widely utilized under the impression of its consistency of results, it has seemed desirable to present our experience with it.

Despite the multiplicity of investigations in this field, there is a surprising dearth of information concerning the stability of this reaction in the individual subject and under comparable conditions. In a study of two normal subjects with blood samples taken every few minutes, Bock, Schneider and Gilbert (1) found that on duplicate determinations there was a difference of 25 mg. per cent in one and 62 mg. per cent in the other at coincident points on the glucose tolerance curve. Glassberg's (2) series on 5 patients showed an average variation between two readings extending from 13 mg. per cent in a fasting state to 40 mg. per cent one hour after the ingestion of glucose. John (3), in a study of the glucose tolerance of children and adolescents, stated that the curve was stable insofar as the normal values did not, on repetition, exhibit the characteristic features found in diabetes mellitus. However, the three subjects on whom graphs were shown had differences at coincident points ranging from 10 to 45 mg. per cent. In a later paper (4), the average variation between duplicate readings one hour after the ingestion of glucose was 47 mg. per cent. Lennox (5) investigated the consistency of the glucose tolerance curve in 50 subjects, most of whom were epileptic; he gave detailed figures on 31 of these. His results also indicate wide variations, although of a slightly lesser extent than those of John (4). Hale-White and Payne (6), in their study on 8 normal young adults, found that there was considerable variation in the same individual on different days, as shown in the rate and height of the rise of the blood sugar and the time taken for it to return to normal.

None of these investigations was performed on individuals on standardized diets and it is fair to assume that, with the known influence of dietary factors upon the type of curve, the variations found in the previous instances might have been lessened by the use of such diets. Hosters (7) stated that he did obtain consistent results in cases of diabetes mellitus on a fixed diet. On the other hand, Nielsen (8) performed 3 to 4 glucose tolerance tests on each of 8 non-diabetic individuals on a uniform diet and noted that the successive tests were on the whole fairly uniform with regard to approximately the same rise but that they differed rather markedly in the time of the rise. In addition, Soisalo (9) reported that in 17 healthy young subjects on a uniform diet, repetitions of the glucose tolerance test showed good agreement in 9 instances and marked divergence in the other eight. His conclusion was that the variations were as great after a standardized diet as otherwise.

The material to be presented in this report includes the glucose tolerance curves of 35 physically healthy male individuals. Thirty of these subjects suffered from schizophrenia but as the values did not differ appreciably from those of the other five normal subjects, nor from the figures obtained from the literature, it may be assumed that the results can be applied to non-psychotic normal individuals. All bloods (venous) were taken in a fasting state with the subjects lying quietly in bed. After a control sample of blood was drawn, 100 gm. of glucose dissolved in a glass of water was ingested. Blood was then obtained at periods of 30, 60, 120 and 180 minutes later. The tests were repeated in 28 of the subjects one week later and in the other 7 at intervals varying from 2 to 80 weeks. The average interval was 4.7 weeks. The diet was not standardized but it varied little from week to week in the institution. The analysis of the blood for sugar was determined by the Folin-Wu (macro-alkalin-copper tartrate-colorimetric) method.

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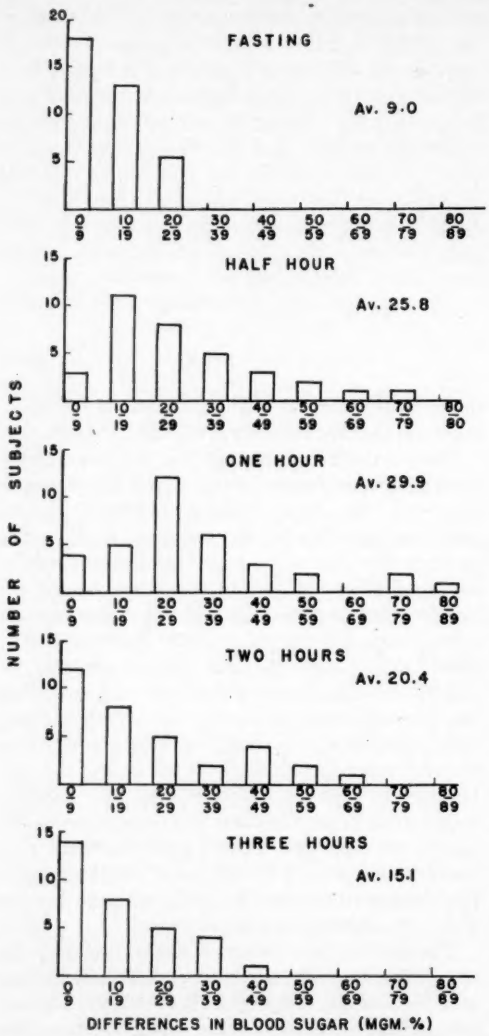

Fig. 1. Distribution of differences between blood-sugar values obtained in two glucose tolerance curves at the fasting levels and one-half hour, one, two and three hours after the ingestion of glucose; 35 subjects.

Table 1 shows the average figures for the two glucose tolerance tests on the 35 subjects. The values are in general comparable to those found in the literature in normal individuals. The mean values for the second test tend to be slightly lower, a trend which has been previously noted by Lennox (5). However, the difference is not sufficiently marked to have any great significance.

Despite the general similarity of the mean curves there are marked variations between the individual pairs of tests. The average differences between similar points on the two glucose tolerance curves are seen in table 2. For the sake of comparison the values obtained by John (4) in adolescents and by Lennox (5) in normal and epileptic subjects are shown. In our series the patients and the normal subjects showed essentially a similar degree of variation. It was least (less than 10 mg.) on the control sample, increasing to 31 mg. at 1 hour after the ingestion of glucose and then decreasing to 15 mg. after 3 hours. The values obtained by Lennox (5) show greater variability than do ours, and those noted by John (4) show the least consistency of all. In their case, it is possible that the longer interval of time elapsing between the two tests may have resulted in the greater magnitude of the differences, although no relationship was observed between the extent of the individual variations and the duration of time in each of the three series.

The distributions of the values of the present series are shown in figure 1. The differences between the blood sugars obtained at similar times in the two tolerance curves have been divided into 10-mg. classes. As one can see, the closest distribution of values is at the fasting level where, in 18 subjects, the

Table 1. Mean blood sugar values of two glucose tolerance tests in 35 subjects

	Fasting (mg.%)	.5 Hr. (mg.%)	1 Hr. (mg.%)	2 Hr. (mg.%)	3 Hr. (mg.%)
First Test	90	138	136	109	92
Second Test	89	131	133	102	87

Table 2. Average differences between similar points on two glucose tolerance curves in non-diabetic individuals

		No. of Cases	Interval Between Tests (weeks)	Differences Between Values at Similar Points in Two Glucose Tolerance Curves				
				Fasting (mg.%)	.5 hr. (mg.%)	1 hr. (mg.%)	2 hr. (mg.%)	3 hr. (mg.%)
	John	14	28	18.9	32.3	47.3	35.8	29.6
	Lennox	31	14	12.8	29.9	41.9	35.9	
Authors	Patients	30	5	9.3	25.5	31.1	20.3	15.2
	Normal Subjects	5	1	7.0	27.4	22.4	20.4	15.0

differences between the two readings is no greater than 9 mg.; in 13 others between 10 and 19 mg.; and in 3, from 20 to 29 mg. One-half hour after the ingestion of glucose, the differences begin to extend to higher levels, reaching the most marked divergence at the 1-hour reading. At this time the most frequent variation between the two determinations lies between 20 and 29 mg. per cent. Subsequently, as the blood sugar levels fall their variability tends to decrease, although the 3-hour reading is still less consistent than the control values. It is evident that, at the lower levels of blood sugar, an individual may show a variation up to 30 mg. (fasting) and under the stimulation of glucose ingestion, the inconsistency of the determinations may be as great as 90 mg. On this basis the reliability of any single value is slight.

The variation shown in figure 1 is not the result of a consistent upward or downward trend in the values. The differences are of a random nature as indicated by the general similarity of the two mean glucose tolerance curves in table 1 and also by the scatter diagrams in figure 2. Here is shown the degree of relationship between the blood-sugar figures obtained in the two tests at the fasting level and at intervals of one-half hour, one and two hours after the ingestion of glucose. If the correspondence of one value with the other were of a high degree, the dots would scatter in a narrow zone from the lower left to the upper right. Of the four correlation graphs only the one representing the 1-hour reading shows any definite trend and this a not very good one owing to the wide scatter. In order to determine whether this relationship could be improved by taking into consideration the fasting level, the individual increases in blood sugar from the control to the one-hour reading in the two tests were plotted against each other. This procedure did not improve the trend. Nor was it affected by taking this increase as a percentage of the control value. It is obvious, therefore, that any prediction that a given value will recur at a similar time in the glucose tolerance curve has a poor chance of fulfillment.

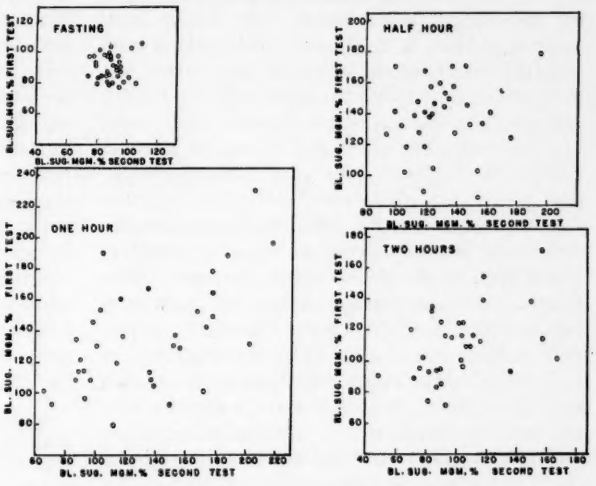

Fig. 2. SCATTER DIAGRAMS to determine the relationship between the values obtained in the first glucose tolerance test and those in the second test at the fasting levels and one-half hour, one and two hours after the ingestion of glucose; 35 subjects.

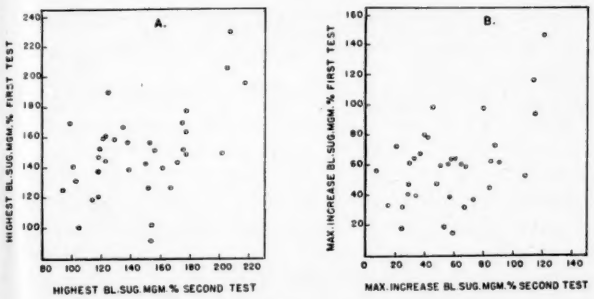
A.
HIGHEST BL. SUG. MGM. % FIRST TEST
HIGHEST BL. SUG. MGM. % SECOND TEST
B.
MAX. INCREASE BL. SUG. MGM. % FIRST TEST
MAX. INCREASE BL. SUG. MGM. % SECOND TEST

Fig. 3. SCATTER DIAGRAMS to determine the relationship between the highest values of blood sugar obtained in two glucose tolerance curves (*A*) and the maximum increases from the fasting levels in the two curves (*B*); 35 subjects.

Since, to the clinician, the maximum level of blood sugar attained may be as important as the time at which it occurs, such maximum values were plotted against each other irrespective of their exact timing (fig. 3, A). The relationship was again very slight. Nor was the situation appreciably improved by taking into account the variation of the fasting blood sugar as in figure 3, B. Here the rise in blood sugar from the control to the maximum level in the first test was correlated with the similar figure in the second test. A marked scatter and only a slight trend was the result. The plotting of percentage increases from the fasting levels again failed to change the picture.

It is evident, therefore, that the variation in the blood-sugar curve following the ingestion of glucose is too great to be of any precise diagnostic value whether one takes identical points, maximal values or increases from the control levels. One must interpret with caution any changes following medication or treatment. It may be argued that such great variation may be attributed to the fact that the subjects were not on a standardized diet. The failure to obtain greater consistency on a fixed diet by Soisalo (9) and the fact that the institutional diet in this series was quite uniform from one week to the next casts some doubt upon the validity of this criticism. Moreover, the extent to which this is true necessarily complicates the procedure and in addition does not eliminate other reasons for the lack of consistency such as the variation in gastro-intestinal absorption and in the pancreatic and hepatic reactions (10) in the ingested glucose.

SUMMARY

The oral glucose tolerance test was investigated as a possible diagnostic feature for endocrinopathic conditions. Repetitions of the procedure showed marked inconsistency in the curves. A review of the literature revealed that there had been few investigations in normal subjects of the consistency of this test. The material was therefore enlarged to include a total of 35 physically healthy male subjects in whom duplicate determinations were made within a short time. Analysis of the data showed that the average variation in the fasting level of blood sugar between the two tests was 9 mg. per cent and extended to 31 mg. per cent at the 1-hour reading with a maximum deviation at this point up to 90 mg. per cent. The relationship between the fasting, half-hour, 1-hour and 2-hour readings, respectively, in the two tests was very slight, the 1-hour reading showing the highest covariance. This inconsistency was not improved by comparing either the maximum levels attained in the two tests or the maximum increase in the blood sugar, irrespective of time. The variability of the test is so great that it has slight diagnostic value except in diabetes mellitus.

We wish to express our thanks to Miss Anna I. Walsh and Miss Cora G. Dyer for their careful determinations of the blood sugar values.

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Comparison of Effectiveness of Various Methods of Administration of Insulin

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The efficacy of the administration of insulin by various routes has been investigated repeatedly. Insulin has been given orally (1, 2, 3), in solution, by instillation into the duodenum, jejunum, and ileum (4), and orally in alcoholic solution within keratinized capsules (5). Murlin's studies (6) showed that on the average 10 units of insulin given subcutaneously had an effect which approximated that of 800 units given orally. The effect was not always proportional to the dose. Komisarenko, et al. (7) demonstrated that insulin was inactivated in the stomach in 15 minutes, in the duodenum in one hour, and in the colon in 2 hours.

Insulin also has been given intranasally (8), by inhalation of insulin spray (9, 10) by rectum, by vagina, and by absorption through the scrotal sac (11). Inunction also has been tried (12). By all of these methods insulin has had weak, doubtful, or frankly negative effects. Consequently, in clinical practice the subcutaneous or intravenous route of administration has continued to be used to the exclusion of all other methods.

Two methods remained which appeared to show a little more promise, namely, that of sublingual administration and of implantation. Preliminary experiments by Sacks (13) suggested that further studies of the sublingual method might be desirable. Certain steroid hormones have been given in this way with good effects.

Parkes and Young (14) in experiments on rabbits found that the hypoglycemic action of amorphous insulin pellets was only slightly more prolonged than that of a similar amount of insulin solution administered subcutaneously. Crystalline insulin pellets had a similar duration of action despite the fact that the tissue capsule around the pellet contained appreciable amounts of insulin.

Using depancreatized dogs, Mark et al. (15) found that pellets of crystalline zinc insulin with protamine were effective for as long as 100 hours. On examination 4 hours after implantation, the pellets were swollen; there was edema at the site of implantation.

Cutting et al. (16) reported briefly on the effective use of pellets of cholesterol and insulin in depancreatized dogs. They observed a slow release of insulin during periods of as long as 13 days. The irregularities of absorption were too great to justify the trial of such pellets in the clinical treatment of diabetes.

This paper is a summary of experiments performed to determine the effectiveness of the sublingual administration of insulin, of the implantation of pellets made of insulin and cholesterol mixtures, and of the implantation of silver cylinders packed with crystalline insulin and open at the ends.

Sublingual Administration

Methods. Experiments were made on 3 normal adult human subjects, one diabetic human being, and two normal adult dogs, to determine the effectiveness of this method of giving insulin. Blood sugars were determined by the Somogyi modification (17) of the micro-Shafer-Hartman method.

The diabetic patient was carefully regulated with insulin and was fed a constant diet which was weighed to the fraction of a gram on balance scales. Food values were calculated. Previous blood-sugar levels in this individual had been 148 mg. per cent 2.5 hours post cibum; 133 mg. per cent 4 hours post cibum; and 187 mg. per cent, fasting. He was given food supplying constant quantities of available glucose at each corresponding daily feeding at 8 and 10 A.M., at 12 noon, and at 2 P.M. He received no insulin on 2 days; sublingual amorphous insulin solution on one day; and 2.0 mg. and 4.0 mg. of crystalline insulin (sublingual) on 2 days. On the day preceding the last sublingual dose of insulin he received 11 U (0.5 mg.) of crystalline insulin subcutaneously. Blood sugars were taken at 8, 9:15, and 10 A.M., 12 noon, and 3 P.M. daily. Insulin was held against the sublingual area in an especially designed glass spoon. The spoon was held firmly against the tongue so as to prevent the mixing of more saliva than was sufficient for solution of the insulin. An area of 2.5 sq. cm. was ex-

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TABLE 1. SUBLINGUAL INSULIN ADMINISTRATION TO DIABETIC PATIENT

Blood glucose, mg. per 100 cc.

Time	4/27/42	4/28/42	4/29/42	4/30/42	5/1/42	5/2/42
8:00 A.M.	103	100 *	96	96 **	96 ***	96 ****
9:15 A.M.				115	107	124
10:00 A.M.	115	124	120		72	112
12:00 N.	96	100	96	92	83	92
3:00 P.M.	100	96	96	96	92	

* 25 U of amorphous insulin, sublingual
** 2 mg. of insulin crystals, sublingual
*** 11 U of crystalline insulin solution, subcutaneous
**** 4 mg. of insulin crystals, sublingual

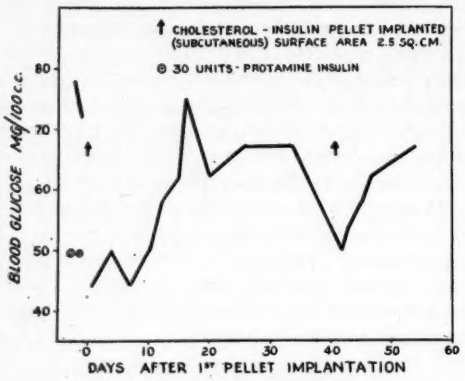

FIG. 1.

posed to the insulin for a period of 10 to 15 minutes.

In the case of the normal human subjects, blood sugars were determined at hourly intervals during a 4-hour period when the subject was in the post-

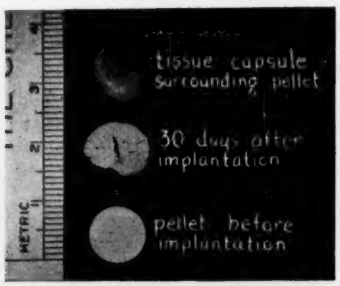

FIG. 2.

absorptive state. In the dogs the blood sugars were determined one-half, 1, 2, and 3 hours after the administration of insulin. In two experiments on the dogs a solution of 20 per cent saponin was held against the sublingual area for 5 minutes immediately preceding the insulin application (1.2 mg. in 1 cc. water). In two experiments the insulin and saponin were applied simultaneously.

Results. Table 1 summarizes the results on the diabetic patient who showed a mild, though distinct, response to the subcutaneous insulin and no response to the doses given under the tongue. The normal human subjects and the normal dogs also showed no appreciable response to sublingual insulin. In the dog there was an unusually copious salivary secretion, particularly in the experiments using saponin. The use of saponin had no significant effect.

Implantation of Insulin-Cholesterol Pellets and Silver Cylinders Packed with Crystalline Insulin and Open at the Ends

The effective use of pellet implants of different hormone preparations suggested an investigation of this mode of administering insulin.

Methods. Normal adult dogs maintained on a constant diet were used. They were fed at 3 P.M. daily.

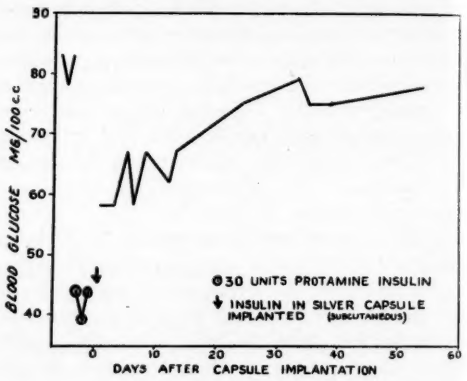

FIG. 3.

All excitement was carefully avoided. Blood was drawn from the saphenous vein when the dog was in the postabsorptive state. Normal blood sugars were determined at 2-hour intervals during the day, and at the same times following the subcutaneous injection of amorphous insulin. Doses of 4, 6, 8, and 12 U were given on different days to determine the response to varying amounts of the hormone. Protamine-zinc insulin also was tested.

After these preliminary tests, pellets composed of a mixture of 20 per cent insulin and 80 per cent cholesterol were implanted. The pellets weighed about 325 mg., had a surface area of 2.5 sq. cm., and were made by tightly packing a mold and hammering the material with 6 or more blows. The fact that the weight range of the pellets was small indicated a constant degree of packing. Silver cylinders (2 mm. in inside diameter × 100 mm., open at the ends) were firmly packed with about 30 mg. of crystalline insulin. In studies with crystalline albumen it had been determined that approximately 1 mg. of protein is released in 24 hours from tubes of this diameter. The

pellets and cylinders were sterilized by heating at 100° C. for one hour on 3 successive days, and were implanted subcutaneously using local anesthesia.

Results. The implantation of the insulin-cholesterol pellets was followed by a marked decrease in blood glucose. The effect diminished steadily over a period of approximately 3 weeks and may have been completely absent thereafter. The fact that average blood-sugar levels for some time following were lower than the control levels suggested a more prolonged mild action. Figure 1 summarizes the results on one dog and is typical of those on the other animals similarly treated. The fact that the dog responded similarly following the second pellet implantation showed that there was no decrease in sensitivity to insulin. After several weeks the pellets were removed and were found to be surrounded by a capsule of firm tissue which on histologic examination showed a mass of granulation and young fibrous tissue typical of that forming around a nonirritating foreign body. The pellet inside the tissue capsule was moist, had become 'mushy,' and evidently was held in shape by the tissue surrounding it (fig. 2). The material, however, had marked insulin potency.

In one case in which there was no visible fragmenting of the pellet, the dog was dead the morning following implantation, apparently of insulin shock. The pellet was examined and appeared to be entirely firm and whole.

Following the subcutaneous implantation of the silver cylinders filled with insulin into the dogs, the blood-sugar levels fell approximately to those observed following the injection of 20 to 30 U of protamine insulin. Figure 3 summarizes the results on dog *M* and is typical of the results obtained on the 5 dogs similarly treated. In one case the release of insulin was observed for only 2 days after implantation. When the cylinder was removed, the ends were found to be completely plugged with firm fibrin-like clots. After 3 to 4 weeks the cylinders were removed and were found to contain appreciable amounts of potent insulin. On histologic examination the tissue surrounding the cylinders presented a picture similar to that which surrounded the insulin-cholesterol pellet. It seems likely that the relatively large size of the insulin molecule is intimately connected with its lack of transmission through the fibrous capsule.

SUMMARY

1. Experiments were made on 3 normal human beings, one diabetic human subject, and two normal adult dogs to determine the effectiveness of the sublingual method of administration of insulin. Any effect of insulin administered in this way was so slight that this mode would appear entirely unsuitable for therapeutic use.

2. The implantation of pellets of insulin-cholesterol (20 per cent insulin, 80 per cent cholesterol) into 4 normal adult dogs was followed by a marked drop in the blood-sugar level. The insulin release gradually decreased until no definite effect of the hormone, as judged by the blood-glucose level, was observed after 2 to 3 weeks.

3. A similar response was observed following the implantation of silver cylinders firmly packed with crystalline insulin and open at the ends.

4. In the case of both pellets and cylinders, the insulin effect was inconstant.

5. Examination of the tissue surrounding the pellets and cylinders showed a mass of granulation and fibrous tissue typical of that which forms around a nonirritating foreign body. This tissue apparently halted the insulin absorption.

We wish to thank Dr. F. B. Peck of Eli Lilly & Co. Indianapolis, Ind., for the crystalline insulin used in these experiments. We are endebted to Dr. J. Adelstein of the Strong, Cobb and Co., for the preparation of the insulin-cholesterol pellets.

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Diethylstilbestrol for Hemostasis in Functional Uterine Hemorrhage[1]

[Oral Diethylstilbestrol]

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A widespread and, not infrequently, uncritical usage of the non-hormonal estrogen, diethylstilbestrol, has followed its release into commerce by the Food and Drug Administration in the early Fall of 1941. Recently, diethylstilbestrol has been accepted by the Council on Pharmacy and Chemistry of the American Medical Association for inclusion in *New and Nonofficial Remedies*.[2]

The practical value of diethylstilbestrol is enhanced by *a*), its low cost, *b*), the fact that it is active by mouth and *c*), its powerful estrogenic activity. These same factors can render it a harmful therapeutic agent if uncritically employed.

Recently our group reported (1, 2) that moderately small daily doses of diethylstilbestrol, when administered orally during the first half of the menstrual cycle, depressed corpus luteum function. No undesirable remote effects followed the moderate dosages employed by us. However, potentially unfavorable results from large doses, or from prolonged therapy, were envisioned. Diethylstilbestrol bids fair to be of particular clinical value in the treatment of certain functional gynecic aberrations. One of these is excessive or prolonged functional uterine bleeding.

Selection of the best manner of securing hemostasis in this condition often seems to be a difficult task for many clinicians. The relative merits of progesterone, androgenic and gonadotropic principles in this regard continue to be discussed at great length, while relatively little consideration is given to the estrogens. The gonadotropins now available have no hemostatic properties. Progesterone, on the other hand, actually increases bleeding instead of bringing about its cessation (3, 4). Androgenic principles possess hemostatic properties but their use in woman is contraphysiologic and may result in severe depression of ovarian function, virilization, or both. These disturbing sequellae not only are undesirable, but they constitute needless traumatism inflicted by an unnecessary form of treatment.

We believe that the choice of the proper hemostatic agent in excssive or prolonged functional uterine bleeding should not be left to one's vagaries, but should be derived from the application of one of four simple rules. These rules have been developed for the purpose of individualizing treatment. Although exceptions are required occasionally, the general rules are as follows. *a*). For a patient 36 or more years of age, having excessive, prolonged or too frequent uterine bleeding, a complete currettage to rule out carcinoma is obligatory. *b*). For a patient under 36 years of age with qualitatively normal ovarian function (progestational endometrium obtained at biopsy), and yet experiencing excessive, prolonged or too frequent bleeding, we elect to make a complete curettage to identify a submucous fibroid, an endometrial polyp, irregular shedding of the endometrium, or malignancy. *c*). For any gynecologic patient having severe and alarming uterine hemorrhage and grave anemia, we make a thorough curettage for gynecic diagnosis and for immediate hemostasis. *d*). For a patient under 36 years of age who is experiencing prolonged or excessive uterine bleeding, and yet who presents no urgent problem of hemostasis, hemostasis and regulation of flowing are obtained by estrogenic therapy.

As early as 1931 one of us (5) reported the use of hormonal estrogen (estrone) in controlling excessive uterine bleeding. Some six years later this therapeutic measure again was reported and recommended by one of us (3). The report of these latter studies was based upon several years of successful use of estrogens. Since then, members of our group have reported repeatedly (6–9) upon the rationale of employing estrogens to

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[1] Part of the expenses of these studies was defrayed by grants to one of us (E.C.H.) from the Research Council of Duke University and from Ayerst, McKenna and Harrison, Ltd., Montreal, Canada.

[2] Report of the Council: *J. Am. Med. Assoc.* 119: 632. 1942.

check excessive bleeding. Not only can hemostasis be obtained in the majority of instances, but subsequently cyclic bleeding can be secured. We have shown also (4, 9) that estrogens and progesterone administered cyclically and intramuscularly following hemostasis with estrogens (or curettage) may result in the re-establishment of normal ovarian-endometrial relationships. This has been observed to occur in 30 to 40 per cent of patients, our criteria for physiologic recovery being the continuation of cyclic bleeding from progestational endometrium and the subsequent intercurrence of pregnancies.

Recently our group described (10) the use of diethystilbestrol in the regulation of excessive and prolonged uterine bleeding. It was found to be as effective as the hormonal estrogens in this regard. At the present time studies are being made of the results of cyclically administered diethylstilbestrol and anhydro-hydroxy-progesterone with regard to its effects on the cyclic recurrence of bleeding and ultimate recovery of ovarian function. We have reported previously (11) that a schedule of cyclically administered oral therapy embracing hormonal estrogens and anhydro-hydroxy-progesterone insured cyclic recurrence of bleeding, but that the percentage of recovery of ovarian function (17%) was low. Others (12), however, have described more encouraging results from the cyclic use of diethylstilbestrol and anhydro-hydroxy-progesterone. Recovery of ovarian function with this oral therapeutic formula may prove to be of greater magnitude than that reported by us.

During our various studies concerning the effects of diethylstilbestrol upon the menstrual cycle and the anovulatory ovarian cycle some observations have been made upon the hemostatic properties of this substance. The present communication deals with these findings.

METHODS

Fifteen women who had prolonged or excessive uterine bleeding were treated with diethylstilbestrol[3] for hemostasis. Their ages ranged from 16 to 37 years, and averaged 25.1 years.

Diethylstilbestrol was administered orally in total daily amounts ranging from 2 to 6 mg. for 6 days. One patient received 12 mg. during 1 day. If bleeding was considerably checked, or hemostasis obtained within 6 days, the dosage was reduced to 4 mg. daily for 1 week. Then the daily dosage was reduced again to 2 mg. for 1 week providing withdrawal bleeding did not occur. In general, the therapeutic schedule, accordingly, covered a period of 3 weeks. However, the duration of individual treatments varied in length from 10 to 24 days.

[3] The diethylstilbestrol (Estrobene), was supplied by Ayerst, McKenna and Harrison, Ltd., Montreal, Quebec.

The continuation of therapy with diethylstilbestrol following hemostasis was designed to maintain hemostasis and, thereby, to regulate the bleeding cycle. Regardless of whether bleeding was due to estrogen withdrawal, or whether it occurred at approximately the expected time, estrogenic therapy, employing usually diethylstilbestrol, was begun on the 5th day of the next episode of bleeding and continued for 10 days, as a rule in daily doses of 1 mg.

Hemostasis was obtained by curettage if the patient could not tolerate diethylstilbestrol, or if it became apparent during the initial days of therapy that bleeding was not decreasing. Subsequent to curettage the patients were placed on cyclically administered diethylstilbestrol or cyclically injected estrogen-progesterone regime in order to establish and maintain regular bleeding.

The patients in this investigation received no other treatment for hemostasis except diethylstilbestrol.

Studies of the endometria were made by means of biopsies. These were obtained at the onset of episodes of bleeding. In general, the endometrium was not sampled until the first bleeding following hemostasis. Classification of the endometria was made by one of us (E.C.H.).

All patients had complete medical, gynecologic and endocrine surveys, including determinations of the basal metabolic rate and roentgenograms of the sella turcica. Particular attention was given to hematologic values.

DATA

There were 10 white and 5 colored women in the group. Nine were married, 3 of whom were colored. Two patients were aged 36 and 37 years, respectively. Each of them previously had had a curettage for diagnostic purposes as well as for hemostasis.

Basal metabolic rates. Values ranged from +11 to −18 per cent. Only 2 were below −10 per cent. These were −11 and −18 per cent.

Hemoglobin levels. Readings were based on 14.6 gm. as 100 per cent. Those reported just prior to initiation of therapy were distributed as follows: 80 to 89 per cent, 3 patients; 70 to 79 per cent, 5 patients; 60 to 69 per cent, 4 patients; 50 to 59 per cent, 2 patients; 40 to 49 per cent, 1 patient.

Duration of irregularities. These ranged from 3 months to 16 years. The average was 5.16 years. The duration of the episode of bleeding at the time diethylstilbestrol was administered for hemostasis varied from 10 days to approximately 1 year. These were as follows: less than 1 month, 8 patients; between 1 and 2 months, 4 patients; between 2 and 3 months, 2 patients; approximately 1 year, 1 patient.

Therapy for hemostasis on previous occasions. Six of

the 15 patients had had some form of therapy for hemostasis in the past. Three of these had had curettages; 1 had received estradiol benzoate; 1 had received cyclically injected estrogens and progesterone; 1 had had a curettage followed by cyclically administered oral estrogens and anhydro-hydroxy-progesterone. The duration of cyclic bleeding following these therapies ranged in length from 1 to 24 months.

Hemostasis with diethylstilbestrol. Hemostasis was secured in 11, or 73 per cent, of the 15 patients. Bleeding stopped within an average of 4.4 days after initiation of treatment. The days on which cessation of bleeding occurred following initiation of therapy were distributed as follows: second day, 1 patient; third day, 4 patients; fourth day, 2 patients; fifth day, 2 patients; sixth day, 1 patient; eleventh day, 1 patient.

The daily dosages with which hemostasis was secured ranged from 2 to 6 mg. They were as follows: 2 mg., 2 patients; 4 mg., 2 patients; 6 mg., 7 patients.

Bleeding ceased within an average of 3.3 days after initiation of treatment with 6 mg., within 4.5 days with 4 mg., and within 7.5 days with 2 mg. dosage.

Failure to secure hemostasis with diethylstilbestrol. This was encountered in the case of 4 patients. Each patient reacted somewhat differently to therapy.

Patient 1. In this instance the therapeutic schedule was as follows: diethylstilbestrol, 6 mg. daily for 6 days; 4 mg. daily for the next 4 days followed by 2 mg. daily for 10 days. Bleeding checked during treatment with 6 mg. daily, but continued throughout the schedule. Curettage was made on the day following cessation of treatment. The patient was then started on a schedule of diethylstilbestrol, 2 mg. daily for 20 days. No bleeding occurred during this treatment.

Patient 2. The therapeutic schedule in this case was the same as that of *patient 1*. Bleeding checked during therapy with 6 mg. daily. It did not recur during therapy with 4 mg. daily. However, after 5 days of therapy with 2 mg. daily, bleeding reappeared, increased, and returned to its former character. The patient was curetted and was transferred to a regime of cyclically injected estrogen and progesterone upon which regular bleeding of normal proportions was reestablished. This patient previously had been bleeding freely or spotting for approximately 1 year.

Patient 3. The patient entered our service with a hemoglobin of 50 per cent, having been bleeding excessively for 11 days. She was hospitalized and started on diethylstilbestrol, 6 mg. daily. At the end of the second day of therapy, bleeding had not been checked and the hemoglobin had fallen to 30 per cent. Curettement was made and the patient was started on a schedule of cyclically injected estrogen and progesterone.

Patient 4. This patient's basal metabolic rate was −18 per cent. She had been bleeding for 26 days. She was hospitalized and given 4 mg. of diethylstilbestrol on the day of admission. The following day a total of 12 mg. was administered. On the third day, the bleeding not having been checked and the hemoglobin becoming lower, curettage for hemostasis was performed.

Withdrawal bleeding. Five of the 11 patients who stopped bleeding under therapy experienced withdrawal bleeding. The time between cessation of therapy and the onset of flowing ranged from 1 to 3 days, and averaged 1.4 days.

DISCUSSION

In reviewing reports on the clinical use of diethylstilbestrol, one is impressed by the large number of postmenopausal women having normally dormant endometrium in whom uterine bleeding was induced. Relatively few investigators have employed diethylstilbestrol in excessive for hemostasis or prolonged uterine bleeding of the younger age group.

Palmer (13) usually obtained satisfactory results with a small dose (1 mg.) of diethylstilbestrol in controlling uterine hemorrhage. However, he encountered instances in which as much as 15 mg. daily was required for hemostasis. Wilson (14) found that 1 mg. daily caused no improvement in the bleeding of 2 patients. Pratt (15) reported on the use of diethylstilbestrol in 7 women who had excessive bleeding; 1 became worse under therapy; 2 were improved; 4 were satisfactorily controlled. Pratt observed in the case of a woman who had been bleeding for 24 days that oral administration of 5 mg. daily resulted in hemostasis 9 days after initiation of treatment. One of us (16) made the following statement in an editorial on diethylstilbestrol in this JOURNAL: 'The hemostatic effect of diethylstilbestrol upon estrogenic (anovulatory) uterine bleeding should permit a cheaper and more practical regulation of bleeding than was possible with natural estrogens.'

Rather than enter upon a discussion of the mode of action of estrogens in producing hemostasis in functional bleeding, a subject adequately covered in reports by members of our group, it would seem pertinent to draw upon observations made during this investigation for pointers which would be of practical value in the treatment of uterine hemorrhage with diethylstilbestrol.

Diethylstilbestrol apparently acts upon the capillary bed of the endometrium to produce hemostasis. In this regard, the action of the non-hormonal estrogen compares favorably with the hormonal ones.

Relatively small daily doses (2 mg.) administered orally can produce hemostasis, although the time required to be effective is longer than when larger doses are employed. We found that the daily dosage of 6 mg

was 70 per cent efficient in producing hemostasis. This percentage is not as high, perhaps, as we have obtained with injected hormonal estrogens. Our group has not employed diethylstilbestrol intramuscularly. Results from this mode of administration might prove equally as good as those following hormonal estrogens. However, the low cost of therapy and the convenience of the treatment to both patient and physician warrant the continued oral use of diethylstilbestrol.

Doses larger than 6 mg. daily were not employed except in the one instance in which 12 mg. were given during 1 day. We doubt seriously that larger doses would prove much more efficient, or that larger doses would have brought about hemostasis in the four reported failures. We believe that hemostasis obtained in from 2 to 5 days in ambulatory patients with a total daily dose of 6 mg. should prove satisfactory to the patient and physician as well.

The patient, whether out-patient or hospitalized, who is to receive diethylstilbestrol orally for hemostasis should be supervised closely during therapy. The physician should not prescribe this form of treatment to the out-patient without giving careful instructions. Changes in the character of flowing should be observed by the patient. An increase in the amount of bleeding should be reported to the physician. Evidences of poor tolerance to diethylstilbestrol need not be anticipated, necessarily, but may be expected. Patients who respond to treatment should be watched following cessation of therapy in order that possible withdrawal bleeding may be dealt with promptly. If this occurs, additional treatment cyclically administered should be given to re-establish regular bleeding periods and, finally, if possible, recovery of ovarian function.

The only patient who had a basal metabolic rate considerably below normal limit failed to respond to treatment with diethylstilbestrol. This result may be significant since we have found that, in general, patients with a low basal metabolic rate responded better to treatment after stabilization with thyroid substance.

No common denominator for failure was found for those patients who failed to stop bleeding under therapy with diethylstilbestrol.

SUMMARY

The oral administration of diethylstilbestrol, in daily doses ranging from 2 to 6 mg., to 15 women who had prolonged or excessive uterine bleeding, produced hemostasis in 11 individuals in from 2 to 11 days. The average time required for hemostasis was 4.4 days.

Of the dosages employed, that of 6 mg. was found most effective. Hemostasis occurred in from 2 to 5 days with this dosage, averaging 3.3 days. Smaller daily dosages secured hemostasis, but required a longer time.

Dosages of diethylstilbestrol larger than those employed in this investigation, or intramuscular administration, are thought unnecessary in view of the satisfactory results obtained in this study.

The effects of the non-hormonal estrogen, diethylstilbestrol, after oral administration, compare favorably with those obtained by the intramuscular administration of hormonal estrogens in securing hemostasis in excessive or prolonged functional uterine hemorrhage.

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Rationale of Estrogenic Therapy in Functional Dysmenorrhea[1]

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Estrogenic therapy for dysmenorrhea has been endorsed by many investigators. The rationale for its use, however, still is based on theory. Some clinicians believe that menstrual pain is a manifestation of an estrogenic deficiency; others claim that an excess of progestin is the exciting cause. Estrogenic therapy is used generally by those who hold either of these views, either as complemental therapy or as a contraphysiologic approach designed to over-ride corpus luteum function. There is another group of workers who employ progesterone on the basis that the pain results from an excess of estrogen and presumably from a deficit in progestin production.

Systems of therapy based on these theories have been described as yielding varying degrees of relief but, as yet, no satisfactory explanations have been offered of the pharmacologic actions which produce desirable symptomatic responses. The alleviation of symptoms is usually regarded as proof of the correctness of whichever theoretical basis was postulated.

Since a recent study by members of our group (1) included an extensive review and summary of the literature on dysmenorrhea, these will not be repeated. However, Boynton and Winther (2) have but lately stressed the importance of the psychogenic factor in the etiology of dysmenorrhea. Dysmenorrheic patients were treated by them with comparatively small oral doses of an estrogen (estriol glucuronide) during the last half of the cycle. The results of this therapeutic schedule and the control placebo therapy were equivocal.

Our group readily admits the magnitude of the psychic element in dysmenorrhea. In fact, we have hesitated to predicate the existence of a functional endocrinopathy in dysmenorrhea. This present communication offers, however, observations made during a broader investigation of the effects of estrogenic therapy on ovarian and endometrial functions (3–5), which seem to rationalize the employment of estrogenic therapy in dysmenorrhea.

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[1] Part of the expenses of these studies was defrayed from grants to one of us (E.C.H.) from the Research Council of Duke University, from Ayerst, McKenna and Harrison, Ltd., Montreal, Canada and from Schering Corporation, Bloomfield, N. J.

METHODS

For this study 18 women were investigated. Their ages ranged from 16 to 30 years and averaged 23.9 years. They complained of severe grades of dysmenorrhea which began at menarche or shortly thereafter.

Each patient received thorough medical, gynecologic and endocrine surveys including determinations of the basal metabolic rate and roentgenograms of the sella turcica. These surveys revealed no obvious causes for the dysmenorrhea.

Three hormonal estrogens (estradiol, estrone and estriol) and a non-hormonal estrogen (diethylstilbestrol) were employed in treating these patients. Estradiol and estrone were administered intramuscularly; estriol and diethylstilbestrol were given orally.

Estradiol was given during the first half of 7 cycles to 4 patients, i.e., from the 5th to the 14th days inclusive; the dipropionate[2] in total doses of 15 mg. was given during 5 cycles and the benzoate[3] ester in total doses of 6 mg. was employed during 2 cycles.

Estrone[4] was administered to 8 patients during 15 cycles in total amounts ranging from 0.67 to 4.67 mg. (20,000 to 140,000 I.U.). It was employed during *a*), the first half of 11 cycles, *b*), during the last half of 2 cycles, i.e., from the 15th to 24th days inclusive, and *c*), during both halves of 2 cycles, i.e., from the 5th to 24th days inclusive.

Estriol glucuronide[5] was administered to 9 patients

[2] Estradiol dipropionate (Di-ovocylin), supplied by Ciba Pharmaceutical Products, Inc., Summit, N. J.

[3] Estradiol dipropionate (Progynon-B), supplied by Schering Corp., Bloomfield, N. J.

[4] Estrone (Theelin), supplied by Parke, Davis and Co., Detroit, Mich.

[5] Estriol glucuronide (Emmenin), and diethylstilbestrol (Estrobene), supplied by Ayerst, McKenna and Harrison, Ltd., Montreal, Quebec.

during 20 cycles in total amounts ranging from 7,000 to 48,600 oral units. It was given during both halves of 17 cycles; in the first half of 1 cycle and during the last half of 2 cycles.

Diethylstilbestrol[5] was given to 6 patients during 17 cycles. It was given uniformly in daily doses of 1 mg. during the first half of the cycles.

Endometrial biopsies were obtained from every patient prior to initiation of therapy and from a number of them during and after therapy. The endometrial tissue was obtained within the first 24 hours after the onset of bleeding. Classification of the responses was made by one of us (E.C.H.).

The cycles were graded according to the severity of the dysmenorrhea described by the patients. These grades were: 0, no pain; 1+, slight pain; 2+, moderate pain; 3+, severe pain; 4+, very severe pain.

Data upon the relation of the onset of dysmenorrhea to menarche, and upon the kind, severity, and duration of the pain were analyzed.

DATA

Data on the initial onset of dysmenorrhea were secured from 17 of the 18 patients studied. Eleven, or 65 per cent of these 17 related this to menarche, while 6, or 35 per cent, described its origin as occurring from 1 to 4 years after menarche. The age for the menarche ranged from 11 to 17 years and averaged 13 years. The age of the patients at the onset of the dysmenorrhea ranged from 11 to 17 years and averaged 14 years.

The onset of pain occurred on the first day of bleeding in 13 patients, 1 day premenstrually in 1 patient, 2 days premenstrually in 3 patients, and 7 days premenstrually in 1 patient. The onset of pain and of bleeding were concomitant in 8 patients.

The duration of discomfort ranged from 3 hours to 10 days and averaged 3.7 days. The mode was 4 days.

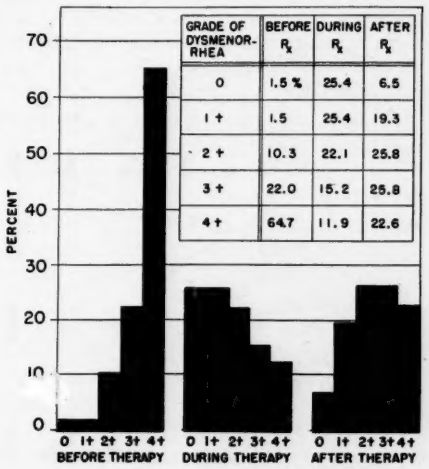

FIG. 1. PERCENTAGE DISTRIBUTION of various grades of dysmenorrhea in the total numbers of cycles studied before, during and after estrogenic therapy.

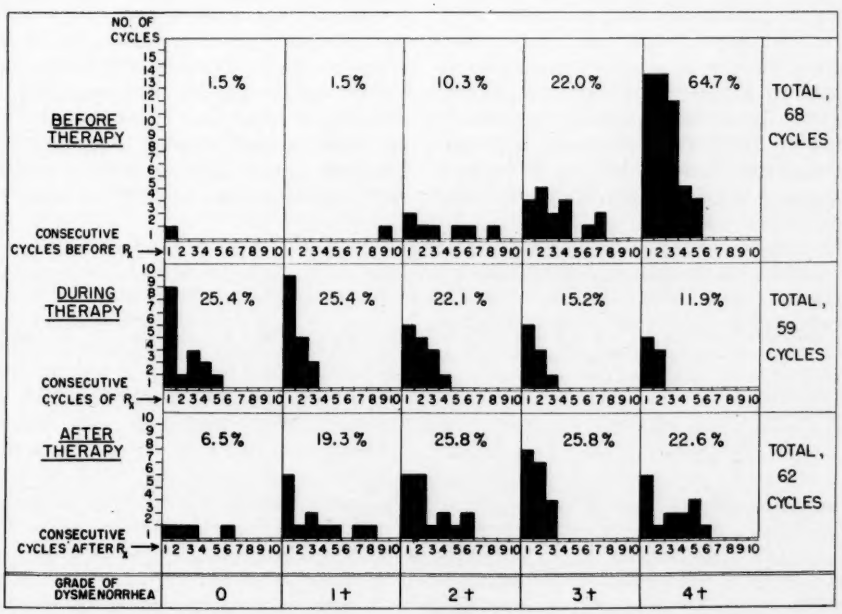

FIG. 2. PERCENTAGE DISTRIBUTION of various grades of dysmenorrhea arranged according to consecutive cycles of study.

In most instances, the pain ended on the 2nd or 3rd day of bleeding.

The most severe pain occurred on the first day of bleeding in 16 patients. The pain was constant and quadrant pain in 1; breast pain in 3; headaches in 3; fatigue in 3; generalized aching in 1; edema, urticaria and diarrhea in 1; nausea and vomiting in 1 patient.

A total of 189 cycles was studied, 68 before therapy, 59 during treatment and 62 following cessation of treatment. The incidence and relative severity of dysmenorrhea in the 189 cycles studied are presented in figure 1. Figure 2 presents the incidence and relative severity of episodes of dysmenorrhea arranged according to consecutive cycles studied. In figure 3 the degree of relief obtained is correlated with the estrogenic therapy employed, with the schedule of its cyclic administration and with the number of consecutive cycles of treatment.

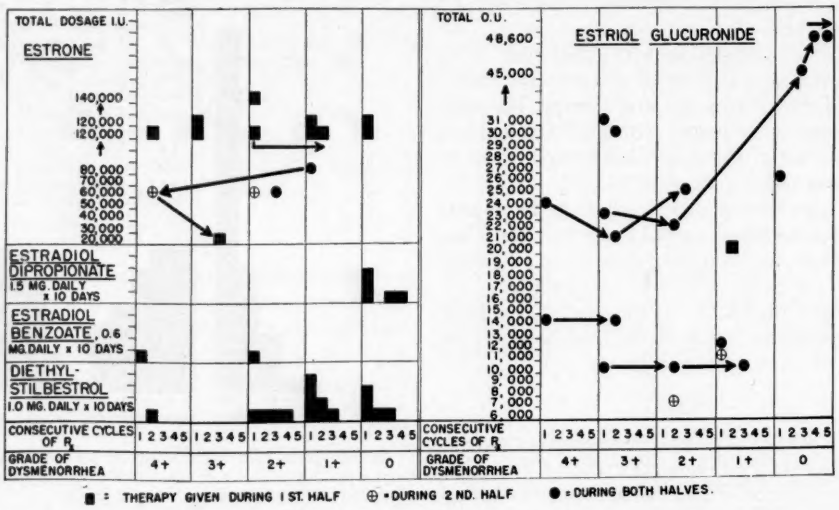

FIG. 3. RELATIONSHIPS OF VARIOUS ESTROGENIC THERAPIES, with regard to dosages and the time of administration during the cycle, and the various grades of dysmenorrhea in consecutive cycles of study. Arrows connect consecutive cycles of treatment and the associated grade of dysmenorrhea of individual patients. Block graphs correlate quantitatively the number of patients who received therapy during the first half of the cycle with the number of consecutive cycles studied.

without marked fluctuations in the remaining 2 patients. Thirteen patients described the pain as being intermittent and cramping, while 5 of them described an aching pain. The pain was chiefly lower abdominal or suprapubic in location in 16 instances and was generalized throughout the abdomen in 2 instances.

Secondary locations of pain and additional menstrual molimina were distributed among the patients as follows: none in 5; low back pain in 7; right, lower

TABLE 1. RELATION OF DYSMENORRHEA TO ENDOMETRIAL RESPONSES

Grade of Dysmenorrhea	0				1+				2+				3+				4+			
Endometrial Response[1]	E	E+	M	P	E	M	M+	P	E	M	M+	P	E	M	M+	P	E	M	M+	P
Therapy																				
Estradiol benzoate										1										
Estradiol dipropionate	3			1																
Diethylstilbestrol	3				3			1	1		1	2								
Estrone						3				2				2			1	1		
Estriol						1				1				1	1			1		
Follow-up after therapy		1	1				1	1	2	4		1	1	3		1				2
Group totals	6	1	1	1	3	4	1	2	3	8	1	3	1	6	1	1	1	2		2
Grand totals	9				10				15				9				5			

[1] Classification of endometrial responses: *E*, moderate estrogenic response; *E+*, marked or hyperestrogenic response; *M*, moderate, irregular (patchy) progestational; *M+*, marked, irregular (patchy) progestational; *P*, normal progestational.

secutive cycles of therapy. Table 1 gives the gradation of dysmenorrhea in relation to the endometrial findings as related to the various types of therapy. All biopsies taken before therapy were progestational in character.

No ill effects resulting from therapy were observed or reported by the patients.

DISCUSSION

It is evident from the data presented in figure 1 and 2 that marked relief from dysmenorrhea often occurred during treatment. This is apparent in a large proportion of the first treatment cycles. These data indicate also that relief from symptoms persisted in a number of instances after discontinuation of treatment. Following cessation of therapy, however, there was a general tendency for the dysmenorrhea to return to the pretreatment level of pain.

While there was relative freedom from pain in 13 per cent of the cycles studied before therapy, there were marked grades of relief in 73 per cent of the cycles during therapy and in 52 per cent of the cycles following the cessation of therapy. Of these, there was complete absence of pain in 2 per cent of the cycles before treatment, in 25 per cent during treatment and in 7 per cent of the cycles studied after treatment.

The dosages of the various estrogens employed and the manner of their administration were apparently definite factors in the alleviation of symptoms. From the data in figure 3 it is apparent that therapy during the first half and during both halves of the cycle was effective in securing relief from pain. These data indicate, but do not show conclusively, that results are more efficacious than when therapy is administered during the last half of the cycle alone. Larger dosages proved more effective than smaller ones. Estradiol dipropionate and diethylstilbestrol were given in the largest dosages and yielded prompt relief from pain in the greatest percentage of treatment cycles. During treatment with estrone and with estriol glucuronide, which were used in the smallest dosages, there was definite evidence of a carry-over and summation of effect in consecutive cycles of therapy.

Therapy in one patient was followed by alterations in the character, time of onset and duration of dysmenorrhea. For 3 consecutive cycles, treatment with diethylstilbestrol was given this patient. Symptomatic relief was noted in every cycle during treatment. Prior to therapy, pain characterized by lower abdominal cramps appeared 3 to 4 hours before flowing and was most severe with the onset of bleeding. Following cessation of therapy, pain described as a severe pelvic ache occurred only on the day prior to the onset of flowing. In the case of another patient, who had received therapy with estrone during 2 cycles, with relief from symptoms, the onset of dysmenorrhea was changed from 1 day prior to flowing to 3 or 4 days premenstrually. There were no other alterations in symptoms. This patient was studied through 6 consecutive cycles following discontinuation of therapy Dysmenorrhea in these cycles was graded as 1+, 1+, 2+, 4+, and 4+, respectively.

That therapy apparently was able to change the character and periodicity of dysmenorrhea is interesting and it seems to indicate a specific relationship. A similar relationship is probable in the case of one patient who reported continued marked relief following the cessation of therapy. During 8 consecutive post-treatment cycles the dysmenorrhea was graded as 2+, 3+, 1+, 1+, 1+, 0, 1+, and 1+, respectively.

The data presented in table 1 show clearly that there is no constant relationship between the type of endometrium from which bleeding occurs, whether estrogenic or progestational, and the presence or absence of dysmenorrhea. These data indicate also that, when relief from dysmenorrhea during therapy was associated with the occurrence of bleeding from an estrogenic type of endometrium, estrogens had been administered in the larger dosage schedules.

This investigation, accordingly, raises the question as to how estrogens act to relieve dysmenorrhea. The concept that relief is brought about by the over-riding of ovulation or corpus luteum function does not seem tenable. An estrogenic endometrial response may be a matter of higher estrogenic dosage and its intercurrence is not necessarily in itself a factor in the relief of pain.

In order to answer this question another question should be faced first. What kind of pain characterizes dysmenorrhea? Dysmenorrhea is, first of all, pain of the sympathetic type. Leriche (6) set up the criteria for this type of pain in contradistinction to the pain of the cerebrospinal type. The latter is fixed and localized and has precise anatomical limits, while the former is more diffuse, of a spreading character, of irregular distribution, readily effecting and affected by the mental state and emotions of the patient. Leriche also showed that pain of either type has a sympathetic component and that, if this be blocked out by surgery or local anesthesia, the pain will be relieved without demonstrable effect on any sensory mechanism in the region where the pain has been.

This being so, and knowing that estrogens relieve dysmenorrhea, what autonomic effects do estrogens have? Reynolds (7) has shown that estrogens have a parasympatheticomimetic action mediated by inhibition of acetylcholine esterase. Here we have a mechanism whereby the sympathetic component of dysmenorrheic pain can be blocked out by antagonistic action of the parasympathetic. From this we may say

that dysmenorrhea is a functional condition brought about by a relative deficiency of estrogen which results in overactivity of the sympathetic nervous system characterized by the production of vasoconstriction and pain.

We are at once faced with the objection that numerous reports in the literature indicate that progesterone as well as testosterone relieves dysmenorrhea. Neither has any known autonomic action. This is true but it is also a fact that both have definite vascular effects and whether the vasoconstriction is overcome by antagonism of the sympathetic or by direct action to produce vasodilation, the end result is the same. Our group has shown (8) that progesterone will cause a fall in blood pressure by arteriolar dilation. Progesterone, testosterone and also desoxycorticosterone given in relatively large doses will produce vascular changes through alterations in capillary permeability (9). It is common knowledge that the vasodynamic drugs exert a beneficial influence on dysmenorrhea. There is even some indication that nicotinic acid, given in doses which produce generalized flushing, will relieve dysmenorrhea.

Progesterone and estrogens administered concomitantly have a very powerful vascular action (9, 10, 11). Gillman (9) has demonstrated that the amounts of estradiol and progesterone required to produce vascular changes in the uterus of the rabbit are inversely proportional, the greater the amount of estrogen used, the less the progesterone required, and vice versa. On the basis of this work, then, an important factor in the production of the vascular changes in the endometrium may be the ratio of estrogen to progesterone.

SUMMARY

Three hormonal estrogens, estradiol, estrone, and estriol and a non-hormonal estrogen, diethylstilbestrol, were employed in the treatment of functional dysmenorrhea of 18 women whose ages ranged from 16 to 30 years. The total dosages of these substances per cycle of therapy ranged as follows: estradiol benzoate, 6 mg.; estradiol dipropionate, 15 mg.; estrone, 0.67 to 4.67 mg.; estriol glucuronide, 7000 to 48,600 oral units; diethylstilbestrol, 10.0 mg.

A total of 189 cycles was studied; 68 prior to therapy, 59 during treatment, and 62 after cessation of therapy. Estrogenic therapy was found to be effective when administered in adequate amounts during the first half or during both halves of cycles. During therapy there was complete absence of pain in 25 per cent and marked relief in 60 per cent of the cycles studied. After cessation of therapy, there was complete absence of pain in 5 per cent and marked relief in 28 per cent of the cycles studied.

No causal relationship was found between the absence or presence of dysmenorrhea and the type of endometrium from which bleeding occurred.

The concept that dysmenorrhea arises as a vascular pain and the mechanisms of pain production and relief were advanced and discussed.

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Clinical Experiences with Oral Ethinyl Estradiol

[Oral Therapy for Menopause]

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TO DATE there have been two reports (1, 2) in the literature of the clinical use of ethinyl estradiol benzoate as replacement therapy. This brief report is to summarize our experiences over a period of twenty-three months in the use of ethinyl estradiol[1] for treatment of the menopause.

SUBJECTS

There were 18 women, all of whom had typical menopausal complaints including hot flashes. No patients were included in this study who did not experience flashes because it was felt that the disappearance of these would more clearly indicate subjective improvement.

The average age was 48 years (range 36–63 years). Two 24-hour urine specimens were collected and assayed for estrogens and 17-ketosteroids. If the results of the assays did not check, additional aliquots were assayed until constant results were obtained. The urines were hydrolyzed, and extracted by the method of Hershberg and Wolfe (3); fractionated by the method of Gallagher et al (4). Specimens were assayed for estrogenic values by injections of an aqueous suspension in divided doses into 15 ovariectomized mice. Assays for 17-ketosteroids were made by the Friedgood-Whidden (5) modification of the Zimmerman method using a Cenco spectrophotelectrometer.

METHOD OF ADMINISTRATION

Both plain and enteric-coated tablets were used. No difference was noted in their effectiveness. Because of toxic reactions occurring during the early days of experimental use of diethylstilbestrol, a very conservative method of treatment was outlined when ethinyl estradiol was first used. After some experience it was found that one .15 mg. tablet three times a day for 3 days, twice a day for 2 days, and then one daily produced no toxic symptoms. The full estrogenic effect was delayed in some patients by this dosage. Further, it was found that in many instances when initial doses of .30 to .45 mg. daily were necessary, the patient could ultimately take .15 mg. every other day and in some instances every third day and keep the symptoms under control. Careful records of the results of treatment extend over a period of from 2 to 13 consecutive months.

To remove the psychological benefits of therapy phenobarbital, grains one-half twice daily, were substituted for ethinyl estradiol. In all instances when this was done, the symptoms returned.

Six cases are reported in detail because of the interesting features encountered.

CASE REPORTS

Case 1, E. S., age 56, married, para 1, grav. 1. Menarche began when the patient was 17 years old. The menses are regular, occurring every 28 days, and are normal in duration and amount. The flashes have increased in intensity over the last 3 to 4 years and the patient has 10 to 12 sweats per day. There has been a definite loss of libido.

Urine analysis showed 5.2 and 3.5 micrograms of estrone, respectively, in the two 24-hour specimens. Values for the 17-ketosteroids were 10.2 and 7.5 mg., respectively for the two 24-hour urine specimens.

Ethinyl estradiol was prescribed as follows: .15 mg. three times a day for 3 days, .15 mg. twice a day for 2 days and beginning on the 6th day, .15 mg. daily. Treatment was begun December 1, 1940. On December 12 the patient reported no improvement and the prescription was changed to .30 mg. daily for 3 weeks. At the end of this time, January 2, 1941, the flashes were reduced in number to 5 a day. The prescription was continued and on February 11, after 40 days of therapy, the patient reported that she was less nervous and that flashes occurred but 3 to 4 times per 24 hours. On February 28, after 8 weeks of therapy with .30 mg. daily the patient reported slight nausea during the day but that the flashes were reduced to 3 per 24 hours. The dosage was reduced to .15 mg. daily on March 21; after 3 weeks of therapy with

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[1] The ethinyl estradiol benzoate was supplied by Dr. Max Gilbert of the Schering Corp., Bloomfield, N. J.

this dosage the patient reported the flashes had increased to as many as 5 to 10 daily and that the nausea had continued. The nausea improved when the medication was omitted for a 2-week period when .15 mg. was again prescribed. On June 19, 1941, the patient reported she was much improved and had gained 24 pounds in weight since September, 1940. On July 31, she complained of soreness of the vagina which disappeared when she omitted the medication for 10 days. She had previously occasionally experienced this irritation when not taking the ethinyl estradiol. The flashes increased in number when medication was omitted. On September 11, 1941, medication was omitted because of soreness of breasts until October 7 when she reported 4 to 6 flashes daily and that the soreness of the breasts had disappeared. Ethinyl estradiol, .15 mg. daily was again prescribed. On April 28, 1942 the dosage was reduced to .05 mg. twice a day. At the last report, May 28, 1942, there were no symptoms of toxicity. In summary, the results in this patient were excellent.

Case 2, M. E., age 47. This patient experienced difficulty in breathing, faintness and a sensation of constriction of the throat. The menses recurred regularly at 30-day intervals, lasting 4 to 5 days; flow normally was quite profuse. The patient reported she was nervous at this time.

In August, 1941, therapy with diethylstilbestrol was started. Severe nausea and 'hives' resulted so that therapy was discontinued.

Urine analysis showed 3.8 and 2.8 micrograms of estrone in the two 24-hour specimens. Values for the 17-ketosteroids were 8.2 and 10.0 mg., respectively.

On December 12,1941, the following schedule of dosage with ethinyl estradiol was initiated. For 3 days, .05 mg. 3 times a day; for 2 days, .05 mg. twice a day, and .05 mg. daily thereafter. Four days later, December 16, the patient reported she felt better generally, but that she was experiencing slight nausea and eructations after taking the medicine for this period of time. She stated she had always had a 'weak stomach.' The dosage was reduced to .05 mg. per day. The next day she developed urticaria. The medication was stopped.

The results in this case were poor. A total dose of .70 mg. of ethinyl estradiol produced urticaria and nausea.

Case 3, L. K. P., age 52. In October, 1939, the patient received radium therapy for metrorrhagia. Six weeks later she experienced hot flashes and increased nervousness. Estrone in dosages progressing from 2000 to 5000 to 10,000 R. U. every other day afforded some relief. In September, 1940, the patient was having 12 to 15 hot flashes daily and was receiving 6000 R. U. of estrone every other day. The physical findings were normal.

Urine analysis showed 10.7 and 12.2 micrograms of estrone in the two 24-hour specimens. Values for the 17-ketosteroids were 7.5 and 7.5 mg.

On the basis of the assay the patient was given testosterone propionate, 25 mg. every 5 days until she had received 6 doses. There was no change in the number of the flashes. Therapy with ethinyl estradiol, .15 mg. twice a day for 25 days was instituted. Marked improvement resulted. Later the patient reported that .15 mg. every other day was sufficient to control the symptoms. As of February, 1942, she reported continued improvement in general health and no flashes.

The results in this case were excellent and there were no toxic symptoms.

Case 4, R. W., age 50. In May of 1940 the patient had received roentgen-ray therapy for uterine fibroids. The last menses occurred in July, 1940, and since that time the patient had experienced hot flashes, excessive perspiration and an increased degree of nervousness and fatigue. Estrone in large dosages had failed to improve the general health or to control the hot flashes. The physical examination revealed nothing of significance.

Urine analysis showed 4.3 and 4.1 micrograms of estrone, respectively, in the two 24-hour specimens. The values for 17-ketosteroids were 6.0 and 7.7 mg., respectively.

On December 12, 1940, ethinyl estradiol, .15 mg. three times a day was prescribed. After 4 weeks of this therapy there was no improvement. The use of other estrogenic substances orally and parenterally likewise failed to alleviate the symptoms.

The results in this instance were poor. Apparently there were no toxic effects.

Case 5, H. W., age 30. For the past 2 years there has been an increase in nervousness, hot flashes, as many as 1 to 3 daily, and a loss of libido. The physical examination failed to reveal any significant findings.

Urine analysis showed 3.3 and 2.2 micrograms of estrone, respectively, in two 24-hour specimens. The values for 17-kesteroids were 5.7 and 6.2 mg., respectively.

On January 22, 1941, the patient was given ethinyl estradiol, .15 mg. daily, until the present time. In December, 1941, the patient reported the flashes had decreased, that she had gained in weight and was less nervous. No toxic symptoms have appeared.

Case 6, M. V. V., age 53. Following cessation of menses 3 years previously, the patient experienced hot flashes and profuse perspiration. Since December, 1941, she has had a marked depression.

Urine analysis showed 10.7 and 12.2 micrograms of estrone in two 24-hour specimens. The values for the 17-ketosteroids were 10.0 and 8.2 mg.

On April 10, 1942, therapy with ethinyl estradiol, .05 mg. three times a day for 3 days followed by .05 mg. twice a day was instituted. The .05 mg. twice a day has been continued. The patient reports she has no hot flashes and that the mental symptoms have improved. What might be considered as a toxic reaction occurred when the patient had taken .75 mg. of ethinyl estradiol. She had become increasingly nervous and nauseated. The dosage was increased somewhat and the nausea was alleviated.

The results in this case are considered excellent.

DISCUSSION

Most of our patients have been treated over a period of months. One toxic reaction was noted. *Case 1* had what might be classed as mild toxic symptoms which disappeared on cessation of therapy but did not recur when ethinyl estradiol was resumed. *Case 2* had an urticarial reaction when only

.25 mg. of ethinyl estradiol was given in a 4-day period. However, diethylstilbestrol had produced the same symptoms. The results in *case 3* demonstrate that ethinyl estradiol can effectively replace theelin therapy. One failure of response to therapy was noted (*case 4*). The report of *case 5* demonstrates the advisability of reducing the dosage as soon as possible with the hope that therapy may be discontinued. The results in *case 6* may demonstrate the possibility of error in concluding that a toxic reaction has been encountered, since continuing the medication in increased dosage alleviated the menopause symptoms and nausea disappeared.

During the period of time in which ethinyl estradiol has been used, no evidence of liver damage or blood dyscrasia has been observed. From our experience it is possible to conclude that ethinyl estradiol is an effective and safe drug to use in the treatment of menopausal symptoms if the patient's tolerance for and requirement of the drug are cautiously ascertained. It should be noted that individual requirements are relatively small for maintaining the patient free of menopausal symptoms and that toxic reactions seldom occur.

SUMMARY

Results of treatment of 18 menopausal patients over a period of 21 months are presented. All patients were under treatment 2 months or more and one for 13 months. Only one patient exhibited what might be considered a toxic reaction to ethinyl estradiol, in that urticarial lesions occurred after taking a small amount of the drug. This patient had had the same reaction to diethylstilbestrol. One must carefully evaluate 'toxic reactions' before concluding that such reactions are not a functional manifestation of a neurotic type. Finally, relatively small doses of ethinyl estradiol are effective in alleviating symptoms of the menopause and with its use uniformly good results have been obtained.

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Studies on Prostatic Cancer

V. Excretion of 17-Ketosteroids, Estrogens and Gonadotropins before and after Castration[1]

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FOR THE PAST several years we have been investigating the subject of cancer of the prostate from the standpoint of its clinical, pathologic, enzymatic and hormonal aspects (1, 2, 3). Fully aware of the difficulties involved in the estimation of gonadal secretion by urinary sex hormone determinations, we undertook a study of urinary hormones in a series of patients with prostatic cancer, before and after castration, in the hope of clarifying secretion-excretion relations and to determine the following: *a*). Are there qualitative and quantitative alterations of urinary hormone excretion in the patient with prostatic cancer? *b*). What effect does castration have on these hormones and is this effect different from that found in the castrate without prostatic disease? *c*). Do extragonadal sources of these urinary hormones come into play following castration? *d*). Is there a relation between the excretory levels of the sex hormones and certain clinical phenomena observed in the castrate?

A review of the literature reveals much work dealing with the urinary excretion of hormones in benign hypertrophy of the prostate including that of Moore et al. (4) and Dingemanse and Laquer (5). However, only a few deal with the hormonal excretion of the patient with prostatic cancer. Dingemanse and Laquer (5) report three cases in which the urinary androgens and estrogens were studied, reporting levels of 14, 7 and 17 international comb growth units per 24 hours for androgen excretion, and 10, 20 and 50 international units of estrogen per 24 hours, respectively, in these three. Satterthwaite et al. in 1941 (6) reported a study of the urinary excretion of total 17-ketosteroids over a period of 6 months in 10 cases of prostatic cancer in which surgical castration was performed in the treatment of the disease. They reported an average close to 5 mg. per 24 hours, and a postcastrational decrease of from 12 to 60 per cent, presumably 6 months after castration. In their group of 10 patients, clinical improvement was correlated with the percentage decrease in the 17-ketosteroids.

With reference to the levels of urinary sex hormones in the castrate and the aging normal male, the authors wish to refer to the excellent works of Kenyon et al. (7), McCullagh (8) and Callow, Callow and Emmens (9); specific reference will be made to these results later in this paper.

METHODS

Collection, preservation and storage of urine samples. In most instances, 24-hour collections of urine were obtained during the entire period of hospitalization which included a 6-day period before operation followed by a 7 to 21-day period after operation. Subsequent 24-hour collections were made at intervals varying from one week to one month subsequent to hospitalization.

Each sample was collected in a bottle, kept in the icebox and, with few exceptions, was extracted within 1 to 2 days. No preservative was used in this study except in a few recent collections when 10 cc. of concentrated HCl was added to the collection bottle. Results indicate that the only advantage of the initial acidification is to permit extraction to be done at a later date.

Hydrolysis, extraction, purification and separation. A few of the initial experiments were done using the Koch method of hydrolysis, extraction and separation (15 min. hydrolysis) (10). Subsequently, however, the method of hydrolysis and extraction of Consolazio and Talbot (11) was adopted; the method of separation described by Koch was retained. This method of hydrolysis and extraction was adopted because of the ease and constancy of steam-hydrolysis and the rapidity with which small volumes can be extracted. Briefly, the preliminary hydrolysis is carried out in an

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hydrolysis chamber employing steam at 95° C. for a period of 20 minutes after acidification of 500 cc. of fresh urine with 50 cc. of concentrated HCl. The temperature of hydrolysis can be uniformly reached in 5 minutes from the icebox temperature. No sample is allowed to boil. Extraction is allowed to proceed for one hour in the Consolazio-Talbott extractor which employs carbon tetrachloride circulating at a rate in excess of 3 liters per hour.

Following the extraction, the carbon tetrachloride is evaporated to dryness over a water bath and the residue taken up in ether. Separation into acid, neutral and phenolic fractions is made in the usual manner by shaking with saturated sodium bicarbonate, 10 per cent sodium hydroxide and 2N sulphuric acid. Decolorization with carbon was tried in a few recovery experiments but abandoned because of loss of active material. After this fractionation, the acid fraction is discarded. The neutral fraction is then evaporated to dryness, dried with absolute alcohol and ether and quantitatively transferred with absolute alcohol to volumetric flasks and later to storage bottles. This fraction is stored in the cold for further separation into *alpha* and *beta* fractions and for colorimetric assay. The phenolic fraction, after acidification with sulphuric acid and extraction with ether, is dried with absolute alcohol and quantitatively transferred through volumetric flasks to storage bottles; later it is taken up in sesame oil for bioassay of estrogens.

Further separations of a number of neutral extracts into ketonic and non-ketonic fractions were made with Girard's reagent *t* according to the method of Talbot, Butler and MacLachlan (12). Many of the neutral fractions and some of these ketonic extracts were separated into *alpha* and *beta* 17-ketosteroids by treatment with digitonin (13).

Colorimetric assay. The 17-ketosteroids were determined in the usual manner employing the Zimmerman reaction as modified by many (14, 15, 16). We adhered to the absolute alcohol technique and used the Evelyn colorimeter. We agree with Friedgood and Berman (16) that a reliable check of the method is had if double the intensity of color is obtained by doubling the amount of urine extract used for the assay. The above colorimetric procedure was also used to determine the *alpha* fraction and to obtain the β-ketosteroids by difference from the total. All results are expressed in terms of crystalline androsterone per 24 hours.

Estrogen assay. Aliquots of the phenolic fraction in alcohol, after quantitative transfer to suitable volumes of sesame oil, were assayed for their estrogen content on 6 to 8 gm., immature, female, albino mice according to the method described by Evans, Varney and Koch (17). These animals were injected twice daily for 3 successive days with 0.05 cc. of the sesame oil solution of hormone. Six animals were used per sample, and 18 hours after the final injection, were killed and the uteri weighed on a torsion balance. At the time of each assay of a large number of specimens, determinations of the uterine-weight response were made on 5 control animals injected with sesame oil alone and 3 groups of 5 mice each injected with pure diethylstilbestrol in sesame oil (0.005, 0.01 and 0.02 μg. The above authors, determining the relative activity of a number of estrogenic substances, found that the relative activity of diethylstilbestrol was 250 per cent when compared to estrone the activity of which was arbitrarily set at 100 per cent. Calculations were made expressing the estrogen output in terms of international units per day using their factor for relative activity and a value of 0.10 μg. of crystalline estrone per one international unit.

Gonadotropin assay. To determine the urinary gonadotropin levels in these patients, the method of extraction of McCullagh and Bowman (18) was used employing fractional alcoholic precipitation. We are satisfied that the authors' conclusion, that this method results in a quantitative, non-toxic extract, is completely justified because rarely did an animal die as a result of toxic manifestations such as cutaneous ulceration. These animals were injected twice daily with 0.5 cc. of the aqueous solution of the hormone for 3 successive days, the same kind of mouse being used as for the estrogen determinations. Six animals were used per extract; two mice received a 1:1 dilution of the 24-hour extract, two a 1:4 dilution and two a 1:16 dilution. The mice were killed 18 hours after the last injection and the uteri weighed.

No standard gonadotropic powder was available for quantitative expression of the results of these determinations. However, quantitative expression was made in terms of diethylstilbestrol equivalents i.e. parallel assays were made using diethylstilbestrol solutions as stimulators of uterine weight. In addition, an expression useful to us alone has been made using the percentage increase in uterine weight over that of uninjected controls.

RESULTS

Range of normal values for 17-ketosteroids. The range of 17-ketosteroid excretion for 10 men between the ages of 20 and 40 was 11.6 to 17.5 mg. per 24 hours with an average of 14.3 mg. per 24 hours. Five women, ages 20 to 35, excreted from 5.6 to 15.5 mg. per 24 hours with an average of 10.1 mg. per 24 hours. These values are in close agreement with those of Albright et al. (19) who reported 13.8 mg. per 24 hours for males and 9.0 mg. per 24 hours for females, which were higher than the values for hospital patients given by Callow and associates (20) of 9.05

mg. per day for males and 6.75 mg. per day for females. Our results indicate, as do those of others (21, 22), that before puberty the values are low (1.1 to 4.2 mg. per 24 hours). The lowest value was obtained in a 3-year-old boy, the highest in a boy age 12 years.

Having established normal values indicative of the reliability of the method, we studied two patients before and after operative procedures other than castration to determine the effect of an operative procedure *per se*. These two patients had hydrocelectomies under spinal anesthesia. *Case 1* showed no change in the excretion of 17-ketosteroids for a period of 20 days postoperatively. *Patient 2*, table 1, showed no consistent change in 15 days. Of added interest is the fact that this patient had a temperature elevation of 101° F. on the third, fourth and fifth postoperative days. Further data, however, are necessary to determine any specific effect of trauma, anesthesia or fever on the output of urinary ketosteroids. We are cognizant of the opposite findings of Albright and associates (19) and shall discuss later the effect of debility on urinary steroid output.

TABLE 1. 17-KETOSTEROID EXCRETION IN THE URINE OF A PATIENT BEFORE AND AFTER A HYDROCELECTOMY UNDER SPINAL ANESTHESIA

Time Since Operation, days	Urine Volume, cc., 1 day	17-Ketosteroid, mg., 1 day
0	670	13.4
1	590	12.9
2	750	19.5
3	460	10.3
4	2670	24.0
5	1260	11.6
6	1590	13.7
7	810	3.5
8	1700	17.0
15	1680	14.9

Levels of urinary 17-ketosteroid excretion in prostatic cancer before and after castration. The excretion of the 17-ketosteroids in 10 patients with prostatic cancer has been studied over a period ranging from 49 to 233 days, 140 individual urinary extractions having been made. In table 2 are shown the results. These values are for total 17-ketosteroids and do not include determinations to be shown for purified ketonic, and *alpha* and *beta* separations. These 10 patients are part of a series reported previously (1) upon whom multiple serum phosphatase determinations as well as roentgen-ray and other studies were made. All of these patients had roentgen-ray evidence of skeletal metastases at some period of their disease. The average value for the group for the 6-day period of observation before castration was 7.62 mg. per 24 hours with a range of 3.6 to 11.7 mg. per 24 hours.

After castration the level of total 17-ketosteroids fell in all cases. As indicated in table 2, the range of the lowest postoperative levels was from 0.4 to 7.4 mg. per 24 hours with an average of 3.49 mg. per 24 hours. This lowest level occurred from 2 to 14 days days postoperatively in 9 cases and at 100 days in one case. The average time of the appearance of the lowest levels for the 10 patients was 15.8 days, but excluding patient *G.T.R.*, the average time was 6.5 days. This fall was not sustained as reported by Satterthwaite et al. (6) but gradually rose in all but one patient (*J.A.Y.*) to a level higher than before castration, ranging from 7.3 to 24.4 mg. per 24 hours and averaging 11.4 mg. per 24 hours. A typical response to castration is graphically illustrated in figure 1 (*Case 3*, table 2).

TABLE 2. 17-KETOSTEROID EXCRETION IN THE URINE OF PATIENTS WITH PROSTATIC CANCER BEFORE AND AFTER CASTRATION

Case No.	Subject	Age	Preoperative Average of 17-Ketosteroid, mg. per day	Lowest Post-Operative level of 17-Ketosteroid, mg. per day	Time Since Castration, days	Highest Postoperative Level of 17-Ketosteroid, mg. per day	Time Since Castration, days	Last Value of 17-Ketosteroid Observed, mg. per day	Total No. of Post-Operative Days Observed	Total No. of Urine Samples Extracted
1	*G.N.A.*	53	3.6	0.4	3	15.9	12	9.4	49	8
2	*A.E.D.*	58	5.7	3.8	14	9.8	233	9.8	233	16
3	*J.D.J.*	58	7.3	5.0	10	10.4	187	10.4	187	15
4	*F.M.*	47	10.8	5.3	3	15.4	27	11.1	183	8
5	*J.H.Q.*	80	5.3	2.1	2	7.3	86	7.3	86	20
6	*G.T.R.*	56	10.8	7.4	100	19.3	4	14.0	128	15
7	*J.T.*	67	11.7	3.1	13	12.3	67	12.3	67	18
8	*J.J.T.*	70	4.2	2.0	3	10.2	115	7.8	183	10
9	*W.W.*	67	7.0	1.6	3	31.4	175	24.4	200	20
10	*J.A.Y.*	71	9.8	4.2	7	7.5	106	7.5	106	10
Average		63	7.62	3.49	15.8 (6.5)[1]	13.95	101.2	11.4	142.2	14

[1] Average time since castration excluding *G.T.R.*

In addition to these 10 patients followed before and after castration, we wish to report the values for 10 patients who were castrated for prostatic cancer, but on whom no preoperative values were obtained. Table 3 shows a range of 1.8 to 16.24 mg. per 24 hours with an average of 6.6 mg. per 24 hours for of the total with an average close to 10 per cent. Five patients showed a considerable fall in this fraction after castration, one a rise and one almost no change. Of the 5 showing a decrease, 4 were observed sufficiently long after castration for the postcastrational rise in total ketosteroids to become manifest. In the

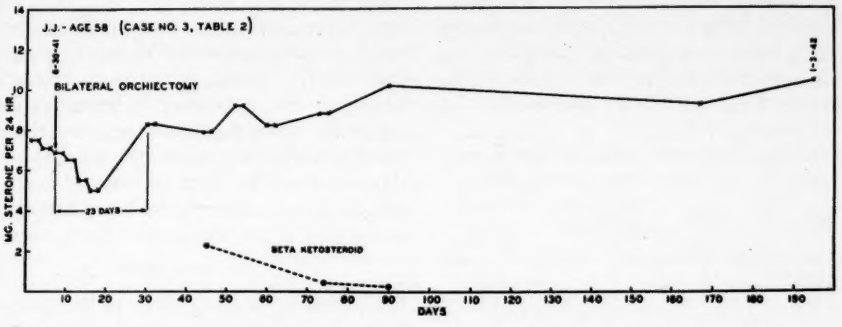

FIG. 1

periods ranging from 7 to 23 months after castration.

Colorimetric values for ketonic and non-ketonic fractions of the total 17-ketosteroids. Separations into ketonic and non-ketonic fractions with Girard's reagent *t* were made on 40 crude samples having a 17-ketosteroid value of 3.7 to 14.0 mg. per 24 hours. At these levels, the range of the ketonic fraction was 89 to 96 per cent of the total (average 94 per cent). Unfortunately, no separations were made of such extreme values as 0.4 and 31.4 mg. per 24 hours.

Levels of alpha and beta ketosteroids before and after castration. Thirty neutral extracts were separated into *alpha* and *beta* fractions. Table 4 lists 21 of the 30 values. The remaining separations were on the urine of castrates upon whom no preoperative studies were made. In these 7 patients, β ketosteroid excretion before operation ranged from 1 to 29 per cent

TABLE 3. 17-KETOSTEROID EXCRETION IN THE URINE OF PATIENTS WITH PROSTATIC CANCER AFTER CASTRATION

Case No.	Subject	Age, yr.	Time Since Castration, months	17-Ketosteroid, mg. per day
11	A.J.	75	23	3.7
12	E.F.K.	64	21	6.9
13	M.M.	56	14	9.14
14	A.Y.	71	7	5.4
15	C.B.	61	12	16.24
16	J.C.	71	10	3.9
17	P.R.	54	14	9.15
18	G.B.	74	9	2.8
19	G.F.	55	7	1.8
20	E.B.	73	15	6.9
Average		65	13	6.6

group without preoperative values, the level of the *beta* fraction never exceeded 8 per cent of the total.

Urinary estrogen levels before and after castration. Multiple determinations of urinary estrogens were made on 6 of the 10 patients described above, both before and after castration (table 5). Before castration

TABLE 4. URINARY BETA-KETOSTEROID BEFORE AND AFTER CASTRATION

Case No.	Subject	Age, yr.	Time Since Castration, days	17-Ketosteroid, mg. per day	Percentage, *beta*
2	A.E.D.	58	0	6.44	11
			13	7.26	5
			103	7.92	5
3	J.D.J.	58	42	7.93	29
			70	8.84	4
			85	10.2	2
5	J.H.Q.	80	0	3.65	11
			5	2.94	11
			17	4.58	3
6	G.T.R.	56	0	10.5	9
			7	13.7	8
			22	12.33	0
			44	13.8	1
8	J.J.T.	70	0	5.33	10
			6	4.5	1
			26	6.47	2.5
9	W.W.	67	0	6.38	2
			15	7.4	5
10	J.A.Y.	71	0	8.58	1
			14	6.4	11
			21	5.15	2

the average excretion for the group was between 12 and 13 I.U. per 24 hours with a range of 1 to 24 I.U. per 24 hours. The last 4 patients illustrated show considerable lowering of the urinary estrogens at the end of the period of observation. Difficult to reconcile with these consistently low values are 3 single postoperative determinations on 3 patients upon whom no preoperative study of estrogen excretion was made. (*Case 2*, 9 and 10, table 2) *Case 2*, *A.E.D.*, excreted 187 I.U. per 24 hours 2 months after castration. *Case 9*, *W.W.*, excreted 110 I.U. per 24 hours 2 months after operation. *Case 10*, *J.A.Y.*, two weeks after

TABLE 5. URINARY ESTROGENS BEFORE AND AFTER CASTRATION

Case No.	Subject	Age, yr.	Time Since Castration, days	Estrogen, I.U. per day
1	G.N.A.	53	0	1.7
			26	15.5
4	F.M.	47	0	7.5
			27	16.2
			41	8.75
5	J.H.Q.	80	0	23.75
			5	4.75
			14	2.5
			17	1.62
			58	2.1
			114	6.0
6	G.T.R.	56	0	18.7
			16	0
			43	9.0
			128	5.8
7	J.T.	67	0	8.5
			5	3.25
			8	1.75
			31	3.75
			72	3.5
8	J.J.T.	70	0	12.5
			3	3.25
			19	8.0
			26	4.2
			46	15.0
			87	11.5
			135	2.6

operation, excreted 78 I.U. per 24 hours. These three levels, all postoperative, were the only ones in the carcinoma group which exceeded 24 I.U. per 24 hours either before or after castration. Using the same method of extraction and assay, we found the range in normal females, ages 20 to 35 years, to be 90 to 150 I.U. per 24 hours.

Urinary gonadotropins before and after castration. Gonadotropic hormone was present in the urine of all men with carcinoma of the prostate. Three patients (*Case 1*, 5 and 7, table 2) were followed for 3, 4 and 5 months after castration. Preoperatively, these 3 subjects excreted quantities of gonadotropic hormone sufficient to produce from a 125 to 200 per cent increase in uterine weight when compared with the uninjected controls. Measured in diethylstilbestrol equivalents per day, this ranged from 0.55 to 0.65 μg. per 24 hours. After castration, at the end of the period of observation, all showed an increase in gonadotropic excretion. Although moderate, this increase ranged from 5 to 80 per cent, i.e. a 5 to 80 per cent increase in uterine weight above the increase observed preoperatively. The most striking rise was observed in a patient, age 63, with prostatic cancer who died of a pulmonary embolism 14 days after castration. Three days after castration, this man was excreting sufficient gonadotropic material in the urine to cause a 500 per cent increase in mouse uterine weight over the controls. Before operation, the value in this patient was 160 per cent.

DISCUSSION

Our findings with reference to the quantity of 17-ketosteroid excreted by patients with prostatic cancer before castration indicate: *a*), that the excretion of these substances is usually lower than in the normal male 20 to 40 years of age; *b*), that these patients excrete about the same amount of 17-ketosteroid as do normal males, age 60 to 70 years; *c*), that the values for these patients agree nicely with the value for castrates given by Callow et al. (20). These workers studied a series of 10 males, ages 19 to 46 years, who had been castrated 1 to 22 years before and found a 17-ketosteroid excretion ranging from 3.1 to 10.9 mg. per day with an average of 7.8 mg. per day. Our precastrational average, as indicated in table 2, was 7.62 mg. per day.

Although the initial levels in our patients are low, there is a further decrease in excretion of these steroids after castration. Two factors may be responsible for this decrease: *a*), removal of the testes may lead to a further reduction in urinary activity, *b*), operative trauma and anesthesia may be responsible for the lowering.

However, shortly after this initial fall in urinary steroids, a gradual, steady rise occurs to a level 50 per cent above the precastrational value. How long this secondary rise continues has not been determined. It would appear from the results on the 10 patients without preoperative values (table 3) that in 7 to 23 months the precastrational level is again approached, indicating a secondary fall in 17-ketosteroids. Further determination on the group on whom multiple determinations were made may fill in this gap.

The explanation of the postcastrational rise in 17-ketosteroids is difficult. Two factors may be responsible: *a*), a deficiency in gonadal secretion may be compensated for by increased activity of the adrenals;

b), an improvement in the patient's general condition may lead to an increase in steroid production and excretion. This second factor would be accomplished by some organ such as the adrenal which ordinarily contributes a large part of the urinary steroids. Evidence to support the first factor, i.e. reciprocal relations of the adrenal cortex and testes, has been advanced by a number of workers including Hamblen et al. (23) and Callow et al. (20). Hamblen and associates, studying the 17-ketosteroids in the urine of women past the climacteric, concluded that an increased production of androgens by the adrenal compensates for regressive changes in the aging sexual system, or, in his words 'that the adrenal becomes the gonads of the aged.' Callow et al. (20) thought that dehydroisoandrosterone might be identified as the characteristic excretory product of the adrenal cortex. If this were true, it would be possible to determine such a reciprocal relation of adrenal cortex and testes. They report one case in which the urine of a eunuch was found to contain an increased proportion of dehydroisoandrosterone over that in normal males. It will be noted that in our series, following castration, there was a consistent decrease in the *beta* fraction not an increase, indicating that the secondary 'compensatory' rise in 17-ketosteroids cannot be explained on the basis of dehydroisoandrosterone and would indicate further that it is improbable that dehydroisoandrosterone is the characteristic excretory product of the adrenal cortex.

With reference to the qualitative nature of ketosteroid excretion in prostatic cancer, we can only state that the *beta* fraction is about 10 per cent of the total, a figure which agrees nicely with that given by Talbot and Butler for the adult male (12). These investigators found that the average daily excretion of α-ketosteroid paralleled the total output while values for the *beta* fraction were found to range from 8 to 15 per cent of the total. This, they indicated, was in close agreement with the value of 10 per cent *beta* found by Butenandt by actual chemical isolation.

Estrogen levels in the aging individual with prostatic cancer are low and there is a further decrease after castration, at least in the individuals on whom multiple preoperative and postoperative values were obtained. The high, single postcastrational values given are hard to evaluate unless here, as may be the case with the 17-ketosteroids, the adrenals compensate by increased activity when the testes are removed.

Urinary gonadotropins are low in these patients before castration. However, there is a moderate rise in gonadotropins after castration. This moderate increase would indicate that the testes are producing smaller amounts of androgen than in the younger male. Consequently, removal of the testes would lead to less pituitary activity than if the androgen titer were high.

Three specific clinical observations are relevant in relation to the urinary hormonal status of these patients after castration: *a*), 'hot flashes' with or without sweating occurred in 8 of the 10 patients whose urinary hormonal excretion was extensively studied; *b*), estimation of the size of the prostate by digital examination through the rectum revealed a decrease in the size of the gland in 9 of the 10 patients; *c*), every patient lost his libido and 8 in 10 expressed an inability to have a penile erection within two months after castration. To date there has been no return of sexual powers. These phenomena have always been associated with what has been termed 'primary gonadal insufficiency.' Hamilton and Catchpole (24) have found this primary gonadal insufficiency to be associated with a decrease in gonadal secretions and an increase in the quantities of urinary gonadotropins.

It appears that primary gonadal insufficiency occurs after castration of the aging male with only moderate changes in urinary hormonal excretion. It may be that in the aging castrate minor changes such as we have observed are of as much significance as greater changes in the younger castrate. However, our results appear to substantiate the original conclusion of Bringel (25) that a demonstration of sex hormones in the urine can not be regarded as evidence of the functional capacity of the gonads.

SUMMARY AND CONCLUSIONS

Urinary hormone levels are described for a series of patients with prostatic cancer both before and after castration.

Before castration, the level of 17-ketosteroids in a series of 10 patients averaged 7.62 mg. per 24 hours. This level is well below that for normal males of a younger age, but very close to levels reported for normal, aging males and a series of 10 castrates reported by Callow et al. (20). β-Ketosteroids represent approximately 10 per cent of the total 17-ketosteroid excreted.

Castration leads first to a fall in 17-ketosteroids followed by a rise. The duration of this rise has not been determined but probably does not exceed one year. β-Ketosteroids do not account for this increase, because after castration, this fraction falls to a low level.

Before castration, estrogen excretion is low, averaging 12 to 13 I.U. per 24 hours. Castration leads to a decrease in estrogen excretion in 4 of the 6 patients studied. However, single high postcastrational values have been observed.

Castration leads to a moderate rise in urinary gonadotropins, a rise, however, which is slight compared to the values reported for younger castrates.

It appears that signs and symptoms of so-called 'primary gonadal insufficiency' occur with only moderate changes in the urinary excretion of the ketosteroids, estrogens and gonadotropins.

These observations tend to substantiate the original conclusion of Bringel (25) that a demonstration of the sex hormones in the urine cannot be regarded as evidence of the functional capacity of the gonads.

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Simple Easy Bruisability: A Pseudo-Hemorrhagic Diathesis of Probable Endocrine Origin

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While investigating the bleeding tendency in approximately five hundred subjects (1–9), we have encountered a number of patients who bruise easily following slight trauma, yet do not have spontaneous purpura. Easy bruising has been the only symptom in some patients, while others have suffered from various unrelated diseases. We have been impressed by the frequent occurrence in the group of certain constitutional factors which appear to bear some relationship to the etiology of this condition, which may be designated 'simple easy bruisability.'

Easy bruising occurs predominantly in women. It may be present from early childhood and is commonly a lifelong affliction. The skin of patients presenting this complaint is usually fair, thin, and unduly sensitive to sunlight and chemical irritants. The bruises occur most frequently in the arms and legs, in areas readily accessible to trauma and the lesions are seldom larger than 3 to 4 cm. in diameter. The patients usually present at least one bruise and in severe cases may have as many as 20 or 30. When the condition becomes pronounced the ecchymoses appear after such slight trauma that it is difficult to distinguish them from those seen in patients afflicted with spontaneous purpura. This is particularly true in the elderly patient. There are no other symptoms of hemorrhagic disorders, except infrequent attacks of epistaxis and, on occasion, gingival bleeding after brushing the teeth. There is no tendency to post-operative hemorrhage from laparotomy wounds, but excessive bleeding may follow tonsillectomy or the extraction of teeth.

In patients suffering from simple easy bruisability, the basal metabolic rate is frequently low, but clinical evidence of overt thyroid insufficiency is seldom present. Rather, there are symptoms of what might be termed 'dysthyroidism,' i.e. irritability, mental anxiety, nervousness, fatigability, peripheral circulatory disturbances, abnormal distribution of body fat, bone and joint pains and the unusual combination of physical sluggishness associated with mental hyper-irritability. Often the onset of easy bruising occurs following hysterectomy. There is a striking frequency of menorrhagia, and the tendency to bruise may be increased immediately preceding or during the menstrual period, and at the time of the menopause. The state of nutrition of these patients is generally good. There are no evidences of specific vitamin deficiencies. The renal and hepatic functions are usually normal, and arterial hypertension does not occur more frequently than in the general population.

Table 1. Normal values for hemostatic tests

Test	Mean	σ_x	Range[1]
Bleeding time (Ivy) in minutes	3.2	1.4	0.4 to 6.0
Coagulation time (modified Lee and White) in minutes	8.8	2.7	3.4 to 14.2
Platelet count (Rees and Ecker) per cu. mm.	422,000	87,000	248,000 to 596,000
Extracorpuscular volume of clot in per cent (5, 6)	9.1	7.5	−5.4 to 24.1
Prothrombin concentration (Quick) in per cent			70 to 100
Capillary permeability (Dalldorf); number of petechiae with 200 mm. Hg vacuum pressure			0 to 10

[1] The statistical method was used in setting up the limits of normality for the bleeding time, coagulation time, extracorpuscular volume of the clot, and platelet count. One hundred normal subjects were studied. The limits of significance of the data were set at two standard deviations, which includes approximately 96 per cent of the observations. The mean is taken at the point of reference. All measures which were calculated were at least three times their sampling errors. The limits of normality for the prothrombin concentration and capillary permeability were arbitrarily set by direct observation. The prothrombin concentration in 98 per cent of the observations fell between 70 and 100 per cent. The range of the capillary permeability held for 88 per cent of the observations.

Wintrobe et al. (15) found a history of easy bruising in the families of some patients suffering from idiopathic thrombocytopenic purpura. We have elicited no family history of hemorrhagic disorders in patients suffering from simple easy bruisability. The

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latter appears to be an inherited characteristic only in so far as it affects the members of the family who are of fair complexion, whose skins are of thin texture, and who tend to burn or freckle, rather than tan in the sunlight.

Because of recent developments in the technique of investigation of the bleeding tendency, it seemed advisable to subject patients suffering from simple easy bruisability to more careful study than has heretofore been possible (1–11). Normal values for the hemostatic tests employed are given in table 1. The diseases of the liver, in malignant hypertension and nephritis, in aplastic, myelophthisic and pernicious anemia, and in the course of some diseases affecting the skin, such as Cushing's syndrome and the Ehlers-Danlos syndrome. In these diseases there is usually a constant abnormality in the results of the hemostatic tests in spite of the fact that the microscopic appearance of the skin is frequently normal. However, in Cushing's syndrome, histologic examination of the skin (12) may show a loss of tissue in the deeper layers of the corium which allows the larger vessels to be

TABLE 2. RESULTS OF HEMOSTATIC TESTS IN 23 PERSONS SUFFERING FROM SIMPLE EASY BRUISABILITY

Case Number	Age	Sex	Duration	Bleeding Time in Minutes	Coagulation Time in Minutes	Prothrombin Concentration, %	Extracorpuscular Volume of Clot, %	Platelet Count, per cu. mm.	Capillary Permeability, No. of Petechiae
1	23	F	Six months	9.0	11.0	90	10	380,000	0
2	42	M	Three years	3.5	11.5	95	19	310,000	50
3	42	F	Since childhood	6.5	12.5	85	11	660,000	0
4	35	F	Since childhood	3.0	7.5	100	4	520,000	6
5	52	F	Since childhood	3.0	11.0	90	9	380,000	1
6	50	F	Since childhood	3.0	9.0	100	2	550,000	1
7	34	F	Twenty years	3.5	7.0	100	5	540,000	1
8	28	F	Since childhood	16.5	11.5	90	6	510,000	0
9	70	F	Since childhood	4.0	8.0	70	−6	540,000	6
10	35	F	Since childhood	3.0	11.0	85	18	310,000	5
11	18	F	Since childhood	8.5	9.5	90	20	360,000	8
12	42	F	Since childhood	3.5	13.0	90	25	310,000	0
13	33	F	Five years	2.0	7.5	100	6	550,000	5
14	27	F	Since childhood	5.5	9.0	100	15	410,000	4
15	28	F	Since childhood	3.0	7.0	100	35	330,000	0
16	31	F	Six months	7.5	6.0	95	−1	410,000	4
17	55	F	Since childhood	4.0	6.5	100	35	400,000	18
18	53	F	Twenty-six years	2.5	6.0	100	−4	380,000	30
19	60	F	Since childhood	9.0	8.5	80	38	370,000	6
20	30	F	Since childhood	2.5	9.0	90	10	380,000	1
21	63	F	Thirty-three yr.	4.0	7.0	80	14	450,000	0
22	40	F	Since childhood	2.5	9.5	100	10	540,000	0
23	42	F	Since childhood	5.0	8.5	90	0	420,000	0

results of the tests performed on patients suffering from simple easy bruisability are given in table 2. The most significant deviation from the normal was a slightly prolonged bleeding time which occurred in 6 of the 23 patients. The capillary permeability was increased in 3, and the clot retraction was diminished in 4. The coagulation time, prothrombin concentration and platelet counts were within normal limits. It is apparent that the factors responsible for this condition cannot be measured by the best available hemostatic tests. However, it seems probable that simple easy bruisability is caused by a combination of factors including undue thinness of the skin, abnormal fragility of the smaller blood vessels and defective cushioning of the subcutaneous vascular bed.

In addition to simple easy bruisability, ecchymoses may occur after slight trauma in various hemorrhagic diatheses such as thrombocytopenic purpura and hemophilia, in obstructive jaundice and brought nearer to the surface. The capillaries may be increased in number and may show qualitative changes. Likewise, in the Ehlers-Danlos syndrome (13, 14), which is characterized by hyper-elasticity of the skin, hyper-extensibility of the joints and increased friability of the skin and its blood vessels, there may be found a relative absence of the fibrous trabeculae which normally bind the cutis to the deeper structures. We have studied biopsy specimens of skin removed from patients suffering from simple easy bruisability and are unable to report any significant alterations.

Easy bruising is likely to be considered of no significance when it occurs in persons who are otherwise in normal health. On the other hand, it may be confused with true purpura of more serious prognostic import when it is present in patients suffering from malignant hypertension or diseases of the spleen, liver, kidneys, or hematopoietic system. Furthermore,

the tendency to simple easy bruising may cause unwarranted hesitancy to perform necessary surgical procedures. In addition, the ease with which bruises are sustained may be of some medico-legal importance.

Many remedies have been tried in the treatment of simple easy bruisability. Small doses of desiccated thyroid substance have been of benefit in some cases. One patient who had suffered from easy bruisability for 5 years recovered after the use of α-estradiol. Nicotinic acid, cevitamic acid and vitamin K have proved to be of no value. The tendency to easy bruising disappeared spontaneously in one patient in whom the condition had been present for 6 months.

SUMMARY

Simple easy bruisability is found predominantly in women who are of fair complexion, have a thin textured skin, who present symptoms of endocrine dysfunction and frequently suffer from menstrual disorders. It is not due to any defect in the coagulability of the blood or decrease in the number of platelets. It is probably caused by a combination of factors, including undue thinness of the skin, abnormal fragility of the smaller blood vessels and defective cushioning of the subcutaneous vascular bed. Small doses of desiccated thyroid substance may be effective in the treatment of this condition.

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A Personal Note on Methyl Testosterone in Hypogonadism[1]

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THIS ANONYMOUS REPORT by a eunuchoid physician is extraordinary. Since the data for many clinical experiments are obtained from patients who have no scientific training, this report is especially valuable because the observer is a physician trained in endocrinology. Although he does not wish to be identified, he deserves full credit.

Synthetic androgens offer a new horizon in the treatment of the adult eunuchoidal syndrome. This is especially welcome to those patients who have received abundant pituitary preparations without discernible benefit. Many of the claims for the testosterones may have seemed too enthusiastic. While a healthy skepticism is commendable, adherence to this attitude in the face of clinical evidence to the contrary may deprive the eunuchoidal patient of therapeutic benefits which will immeasurably add to his well being and happiness.

Testosterone propionate has been administered by injection (1, 2) and testosterone by subcutaneous implantation of pellets (3). More recently methyl testosterone, when given in sufficient amounts, has been found to be active orally (4, 5, 6). The therapeutic benefits of the oral form, when used in proper dosage, are as great as by injection or pellet implantation.

The majority of the reports on the use of testosterone deal chiefly with the more obvious effects on the external genitalia, while scant attention is paid to the effect of therapy on the patient as an individual. It is for this reason that a report of a successfully treated patient, who is a physician with considerable endocrine training, may be justified.

CASE REPORT

This patient, 32 years old, 6 feet tall, weighed 178 pounds. Aside from the endocrine features, the physical examination was essentially negative. The general appearance of the patient gave the impression he was at least 10 years younger than his true age of 32. The bodily configuration was typically eunuchoidal. The pelvis was broad and feminine. The length of the extremities, especially the lower, was disproportionate to the trunk length. The musculature was poorly developed. The skin was smooth, pale, and devoid of hair except for the hair of the head and a few sparse pubic and axillary hairs.

The external genitalia were infantile. The penis, approximately 2.5 cm. in length, was nearly buried in the puberal fat. A small scrotum contained testes slightly larger than the common bean. They were relatively insensitive to pressure. No prostatic tissue could be palpated.

Routine blood and urine studies were negative. The basal metabolic rate had averaged between −15 and −22 per cent over a period of several years. Extraction of the urine for testicular hormone was consistently negative by a method that yielded an average of 20 bird units per liter in the normal male. Roentgenograms showed the major epiphyses ununited.

The patient had been treated extensively with various gonadotropic pituitary and pituitary-like preparations during the preceding 15 years. At one period he had received as much as 500 R.U. of anterior pituitary-like gonadotropic substance per day for 25 days. He had never shown the slightest response to any therapy.

In August, 1940, the patient was treated with 20 mg. of testosterone propionate in a cream base by inunction. The application usually was made before retiring. Successive regions on the trunk and extremities were utilized to facilitate absorption.

At the end of the first 48 hours the skin of the scrotum, nipples, and penis was noted to be hyperemic. By the fourth day penile erections occurred as frequently as once per hour. After treatment for one month the skin of the the nipples and of the external genitalia, especially the scrotum, had become darkly pigmented. The scrotal skin had become redundant, thickened, and rugose. The penis had doubled in size, partly due to a constant semiturgid state. The testes, apparently not affected by the treatment, remained small. At the superior pole of each testis, a small, slightly tender mass was noted, possibly indicating some development of the epididymis. After about 30 days of treatment the patient experienced a seminal emission. The fluid was colorless and watery, and did not coagulate. The volume was estimated at 0.25 cc.

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[1] The liberal supplies of testosterone ointment (Oreton-F) and methyl testosterone (Oreton-M) used in this study were furnished by Dr. W. H. Stoner of the Schering Corporation, Bloomfield, N. J.

Microscopically the fluid was noted to be composed of mucus and to contain many polymorphonuclear leukocytes.

The few pubic hairs present grew rapidly and many new hair follicles developed. The previous hair was brown, soft, silky, and straight. The new hair was jet black, coarse, and curly. The distribution of the new follicles was feminine. No effect was noted on the hair of the axilla, head or face.

Testosterone ointment produced a general feeling of well being, but sexual drive was lacking. It occurred subsequently with the use of methyl testosterone. There was no especial change in muscular energy or endurance. Careful checks showed no variation in body weight.

During the entire period of treatment by inunction, there was neither the slightest suggestion of skin irritation nor other contraindication to its continued use. There may have been slight urinary frequency.

After about 10 days of treatment, when it was apparent that the therapy was very effective, a photograph of the external genitalia was made. Except for a slightly increased growth of the pubic hair, the basic state before treatment was retained (fig. 1). It will be noted that the genitalia are infantile and correspond to those of a preadolescent boy of under 10 years of age.

During the next 6 weeks (September and October) treatment was sporadic. Several days without treatment resulted in a prompt regression of the turgidity of the genitalia and fewer erections. It was astonishing to observe how promptly the regressive changes appeared when therapy was discontinued.

Methyl testosterone therapy became available during the latter part of October. In order that the effects of the oral preparation would not be confused with the previous therapy, treatment was omitted for a period of about 2 weeks. During this period the size of the external genitalia regressed to approximately the state before the use of androgens. The pigmentation, especially of the nipples and the scrotum, faded considerably. Erections did not occur. There was some diminution of the feeling of general well being.

Initial treatment with methyl testosterone was begun in November. During the first week the dosage was low in order to avoid possible deleterious effects, but none was noted. No unusual effects were noted at a daily dose of below 20 mg. When the dose was raised to 40 mg. there was a prompt occurrence of erections.

During the month of November an average of 40 mg. of methyl testosterone a day was taken orally. This level seemed to produce effects similar to 20 mg. of the testosterone ointment. It was noted early that the erections were rather sharply conditioned by the size of the dose. This correlation was less obvious later in the treatment. Erections, an early sign, were nearly always noted within 12 to 36 hours after the ingestion of the tablets. The patient had a number of seminal emissions during the latter part of the month. Pubic hair and penile growth were rapidly increased. At this period the voice was first noted to assume a slightly hoarse quality. The patient became aware of a slight psychological change.

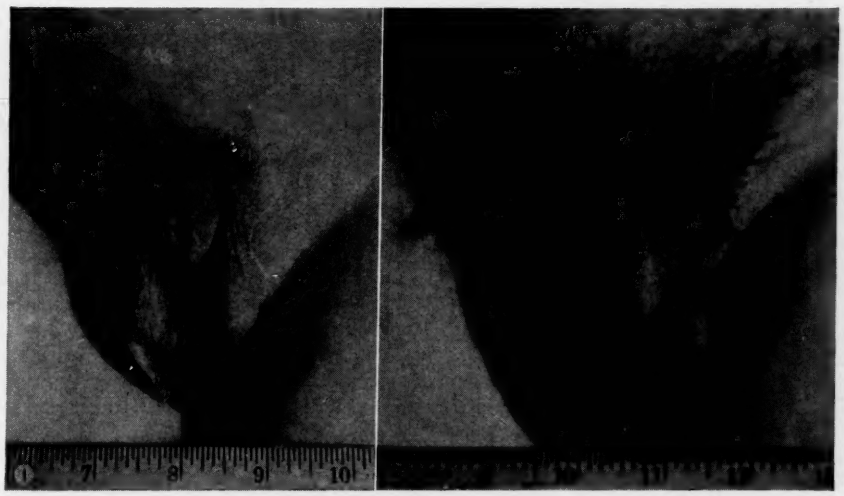

Fig. 1. August, 1940. Fig. 2. February, 1941. Photographs approximately one-half actual size.

The external genitalia progressed as they had with inunctions. Increased pigmentation gradually darkened the skin, especially in the face. The patient had always appeared 'pale,' even when in a state of good health. The pigmentation took place in the winter months when there was almost no sunshine. It was noted by friends who were not aware of the treatment.

The sebaceous glands at the base of the nose became active, giving the nose an oily appearance. This had never been noted before. No change in the function of the sweat glands was observed.

In December, the average dose was raised to 60 mg.

per day, whereupon considerable psychological change occurred. Libido was more marked. The voice had a definite huskiness and was slightly lower in pitch. A striking effect was the breaking of the pitch of the voice as in the adolescent boy. During this month the external genitalia increased in size but not as rapidly as was previously observed. The testes decreased slightly, were softer and less sensitive to pressure. This was not considered by the patient to contraindicate continuing treatment. He was delighted with the results of the treatment and felt better than he had in years.

In January, 1941, the average daily dose was raised to 80 mg. During this month there was a sharp drop in response to the medication. Libido decreased abruptly; the penis became less turgid than usual; the number of erections diminished. The voice continued to be husky. This regression is not readily explained. The observations made during this period served to prove the patient's reaction was not conditioned by his knowledge of the dose.

No therapy was taken for 10 days early in February. When resumed, a daily dose of 50 mg. was taken. The response was reasonably prompt but did not reach a great height. Almost no treatment was taken in the month of March. It was felt that a rest might prove beneficial. An average of about 10 mg. a day was taken during this period. Regression was quite prompt.

Treatment was resumed in April with an average daily dose of approximately 50 mg. By midsummer it was noted that the therapy was less effective. No treatment was taken for a month. When resumed the effect was slight. There had been relatively little increase in the size of the external genitalia, no marked change in hair growth, definitely diminished libido, and frequent seminal emissions. It seemed that while maintenance of the secondary sexual characteristics was achieved through treatment, there had been relatively small progress since mid-spring. Accordingly, as an experiment to determine the effect of increased dosage, 150 mg. (15 tablets) were given at one time, in the evening before retiring. By the end of 12 hours the external genitalia were turgid; it was noted that a full bladder produced priapism. At the end of 24 hours the penis was in a state of semi-erection constantly. Tingling was noted in the nipple. A mammary tree of approximately 3 cm. diameter could be palpated. The nipples were prominent, as is often observed in the adolescent boy. Libido was quite marked. Forty-eight hours after the ingestion of the 15 tablets these symptoms had all disappeared except that the breast tissue continued to be tender for several days. At the end of 72 hours the external genitalia resumed their former appearance except for a slight hyperemia. The patient has repeated the dosage of 150 mg. several times with identical results. Toxic effects have never been noted. No gastrointestinal intolerance has ever been observed. The only effect noted was a very slight increase in the frequency of urination. Nocturia was never reported by the patient.

Psychological Aspects of the Treatment

Much of the value of this report will be found in the effect of the treatment on the mental state and social activities of the patient. It is quite remarkable that a few tablets, taken daily, could so change the behavior and outlook of an individual.

In order better to appreciate the effects of androgenic therapy there must be some degree of insight into the patient's reactions before treatment.

Prior to therapy the patient had only the mildest interest in the opposite sex. He enjoyed a certain amount of social activity and had occasional 'dates,' but only to secure a partner for some social function. He was able to recall about 5 dates in the year before treatment. Contact with the opposite sex was in no way stimulating. The patient rarely indulged in the so-called 'necking' because he felt embarrassed. The patient had never had a seminal emission.

The general behavior of the patient was shy. He frequently over-compensated to the opposite extreme and appeared an unmitigated 'smart aleck.' He felt embarrassed in the presence of strangers because of his high-pitched voice, lack of beard, unmasculine contour, and youthful appearance. No doubt the patient was unduly sensitive regarding these points, but long years of being called 'Madam' over the telephone had sensitized him.

On the whole, the effects of testosterone were stimulation of rapid development of adolescence. The physical and psychological development which normally spreads over a period of years now took place in a few months. In the early part of the experiment, during the use of ointment, libido was very slight, although the physical stimulation was very evident. After the institution of the methyl testosterone libido became a prominent feature. This gradually increased as the treatment progressed; however, the stimulation was never uncontrollable. It should really be considered as a gentle, but nevertheless definite urge, rather than a true drive. Huskiness of the voice has progressed until now the normal speaking tone is much lower. He no longer is subject to telephone difficulties. Friends frequently notice the change and ask whether he has an upper respiratory infection. This single change as a result of the therapy is the most gratifying to the patient of all the responses.

The increased desire for feminine companionship, not based solely on sexual premises, resulted in an almost unbroken succession of 'dates.' The patient came to enjoy 'necking'; close contact frequently resulted in erections and seminal emissions.

Close friends of the patient remarked that the patient seemed 'more mature' and 'emotionally more stable.' He lost a great deal of his shyness, especially with the opposite sex.

The patient was subject to rather severe attacks of depression prior to therapy. These attacks usually

appeared without any known cause and very frequently extended over a period of several days. Since the institution of treatment the depressive attacks have practically ceased and there has been a definite improvement in his general spirits. The patient is definitely more aggressive.

The patient is emphatic in his declaration that the therapy has increased his general feeling of well being, and yet he is unable to give a logical explanation. He appears to make every attempt to be objective in his observations. He reports that when under treatment he definitely felt more pugnacious and was frequently surprised to find himself 'talking back to taxi drivers, and other things I wouldn't have done 6 months ago.'

RESULTS OF THE TREATMENT

The skin is more heavily pigmented, most pronounced in the external genitalia and the nipples. The sebaceous glands are much more active. The increased pigment has resulted in the loss of the patient's chronic 'pasty' appearance. The skin tans more easily when exposed to sunlight, but there has been no change in its general texture. There was no acne.

He doubts if there was any change in his energy output and states he felt just as tired after a difficult day in the hospital as before the institution of androgenic therapy.

The pubic hair was markedly affected by the therapy (fig. 2). Before treatment the pubic hair was limited to a few fine strands; after treatment it enveloped the entire pubis and scrotum and tended to be rather coarse, black, and recently slightly curly. There has been no typical masculine extension toward the umbilicus. However, recently there has been some suggestion that this may occur with further medication. Curiously enough, there was no effect on the axillary hair or hair of the head.

Only very recently (September, 1941) has there been evidence of development of a beard. The lanugo of the upper lip has become heavier and coarser. This is considered to be a promise of eventual development of a beard with further treatment. No change was noted in the hair of the extremities.

There has been no gain in weight as reported by some investigators (3, 7). The patient made careful observations regarding this point, weighing himself daily on the same hospital scale over a period of many months.

The size of the penis has been increased about three-fold but the size of the testes has remained stationary. The epididymides, which before treatment were not palpable, have attained a size approximating that of the atrophic testes. The prostate was not palpable prior to therapy. Very recently the prostate was palpated by a urologist who declared the gland to have two well-developed lobes with a median furrow, and while small, to be within normal limits. The seminal fluid is normal microscopically except for a total absence of spermatozoa. The ejaculate has increased in volume from a few drops to about 2.5 cc. It is turbid and coagulates on standing.

The major epiphyses are ununited. Fluoroscopically no change has resulted from treatment.

Treatment resulted in a fairly marked lowering of the vocal pitch, noted by both the patient and friends. Recently there has been considerable increase in the size of the laryngeal prominence.

DISCUSSION

In this report, which is unique because the patient is also a physician, and functions as such, there are certain aspects of hypogonadal treatment which deserve consideration. The chief cause of concern to the hypogonadal individual is not his lack of sexual interest or ability, for indeed, how can he be concerned about a force of which he knows nothing? The lack of insight into this aspect is the principal mistake made by the average physician in the treatment of the eunuchoidal individual. Of far more concern to the patient are the distinct social handicaps of an effeminate appearance and the high-pitched voice. Thus, while this report deals largely with the more obvious effects of the treatment, the sexual changes, I desire to stress that this phase represents only one link in the chain. So far as the patient is concerned, it is of less importance. Of far greater importance is the rehabilitation of the eunuchoidal patient into feeling that he is able to assume his place in the world as a normal person.

Oral therapy appears to be the choice in adult hypogonadism. Implantation of pellets has many basic difficulties and the dosage is not amenable to regulation (7).

Increased tolerance has been noted by others (4). In the case reported, the tolerance developed over a period of 6 months to the point where a daily dose of 100 mg. was required to produce the response originally noted at 40 mg. Interruption of treatment over a period of several weeks appeared to restore partial sensitivity to the hormone. The sole deleterious effect was the production of a mild gynecomastia which occurred only with larger doses (8).

SUMMARY

1. Oral therapy by methyl testosterone is capable of initiating normal adolescent changes in an adult eunuchoid individual.

2. No toxic effects resulted from massive doses of the synthetic hormone.

3. There is a suggestion that a tolerance to the substance may be developed.

4. Methyl testosterone appears to be the product of choice in treatment of the adult hypogonadal individual.

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Hypothyroidism, Hyperlipemia and Carotenemia

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THE RECOGNITION of the clinical picture of carotenemia is relatively new. In 1904 van Norden described this orange-yellow pigmentation of selected areas of the skin in diabetics and called this disease xanthosis diabetica. That carotenemia was associated with some metabolic disturbance was suggested by van Norden's (1) publication in 1907, and, as further studies appeared in the literature, the correctness of this assumption became more apparent.

In 1919, Hess and Myers (2) described this same peculiar pigmentation of the skin occurring in children and called it carotenemia. They noted that this coloring appeared in two children who had ingested large amounts of lipochrome vegetables, but they were unable to reproduce this condition by the overfeeding of these same vegetables in control cases. Head and Johnson (3), reported the same phenomenon in malnourished children who were deprived of meat but had eaten an excess of pigment-containing vegetables. The carotenemia subsided when this excess of lipochrome was removed from the diet. They were aware that this condition was not universal and that children exposed to the same dietary limitations and excesses did not all develop carotenemia. Stoner (4), in 1928, made the same observation and stated that carotenemia appeared uncommonly in adults and that its occurrence in children is associated with an increased vegetable intake.

Boeck and Yater (5), reporting the occurrence of xanthosis and xanthemia in variously diseased patients, showed that 86 per cent of the diabetics examined had xanthemia and that 100 per cent (of 22 cases) of those who had renal disease were xanthemic. Only 69 per cent of the patients with hepatic disease had xanthemia. In analyzing the cases of 36 patients with xanthemia, it was found that 10 were diabetics, 12 had 'stomach trouble,' 2 were jaundiced and the remaining 12 had indifferent or unrelated complaints.

The metabolism of carotene has been shown to take place in the liver (6, 7, 8), and one could expect an alteration of the blood carotene balance associated with hepatic insufficiency. This, however, has not been demonstrated consistently, perhaps because of the lack of sensitivity of the various laboratory tests for liver function, or because complete studies were not made in all instances. When there is a demonstrable carotene imbalance there is always an associated, but not necessarily proportionate, change in blood lipid mobilization (9–12). What exact mechanism in liver function is altered in carotenemia is not known, but the most plausible explanation is that the liver is incapable of converting carotene to vitamin A (11, 12, 13), possibly because of the absence of carotenase. This results in an excess of carotene over and above the ability of the liver to store the lipochrome and, therefore, the blood serum is flooded with this excess. It accounts for the fact that clinical carotenemia is impossible to reproduce experimentally in normal controls (12).

The association of carotenemia and thyroid dysfunction is not unknown (13–17); and it has been shown that this relationship can be demonstrated in vitro (15). That this relationship should exist is not strange. Carotene is a lipochrome and it is natural to expect that a syndrome which affects the metabolism of lipochromes would also affect the mobilization of other lipoids whether they have a pigment base or not.

Euler and Klussman in 1932 (16) showed that white rats fed with large doses of carotene and thyroxin lost no weight, and Wendt (14) confirmed this antagonism of carotene and thyroxin by successfully decreasing the basal metabolic rate of hyperthyroid individuals by the feeding of large doses of a vitamin A preparation. The increased creatin metabolism of hyperthyroid individuals was shown to be lessened by the administration of carotene (18, 19). Abelin (15) demonstrated that guinea pigs depleted of vitamin A and carotene, showed an absence of these substances in the liver; when the animals were fed carotene both of these substances re-appeared in the liver, but if the diet were supplemented with both carotene and thyroxin instead of carotene alone, then the liver failed to show any storage of either carotene or vitamin A.

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Experiments in vitro, (15), in which a mixture of carotene and thyroxin was allowed to incubate, showed that there is a progressive diminution in the quantity of both of these compounds.

In 1938, Anderson and Soley (20) reported 13 cases of carotenemia of whom 5 were hypothyroid; 3 cases showed evidence of hepatic damage, and one case had both hepatic insufficiency and hypothyroidism. Savy, Vachon and Vincent (21) reported 6 cases of carotenemia, 5 of whom had some hepatic dis-

TABLE 1. RESULTS OF LABORATORY TESTS PRIOR TO AND DURING THERAPY

Test	Before Therapy	One Month of Therapy[1]	Two Months of Therapy[1]	Eight Months of Therapy[1]
Hemoglobin, %	70	81		85
Red blood cells, M./cu.mm.	3.35	3.55		4.35
Color index	1.05	1.10		.98
Total protein, gm. %	7.6			7.49
Albumin, gm. %	5.2			4.77
Globulin, mg. %	2.4			2.72
Albumin/globulin ratio	2.17			1.75
Nonprotein nitrogen, mg. %	33.8			36.0
Total lipids, mg. %	1290.0	994.0		597.0
Phospholipids, mg. %	252.5			
Lipid phosphorus, mg. %	10.1			
Cholesterol, mg. %	628.0	329	201	265.0
Free cholesterol, mg. %	142.0	87	53.3	61.4
Percentage free cholesterol	23	23	26	23
Icterus index	15.1	13.3		
Carotenoids, mg. %	0.448	0.368	0.220	0.266[2]
Basal metabolic rate, %	−20	−5	+9	+1
Body weight, lb.	157	149.5		137

[1] Therapy was continuous throughout the 8-month period and consisted of ferrous sulfate, grains 15, per day and thyroid (whole gland), grains 6, per day.

[2] Average normal blood carotene is 0.109 mg. per cent with a range from 0.054 to 0.176 (10).

turbance, and the other case, reported in detail elsewhere (13), was one of carotenemia associated with hypothyroidism. In a recent paper by Escamilla (22), 7 consecutive cases of hypothyroidism were presented, each having increased carotene pigments in the blood serum. Four cases were treated with thyroid, in 3 of which there was a consequent decrease in the blood carotene levels. His explanation for the carotenemia was a depression of liver function due to a lowered metabolism.

The case to be reported in this paper is similar to those described by Anderson and Soley (20) and Savy et al. (21). The case, however, presents a more detailed study of the blood lipid and metabolic changes, both during the course of the disease and following treatment.

CASE REPORT

M.S., a 36-year-old white male, entered the out-patient department of the Jewish Hospital, on Jan. 28, 1941, complaining of weakness, fatigue and moderate exertional dyspnea of 2.5 years in duration. He stated that there was a change in his physical status about 4 years ago. At that time, he noted that his skin became coarser, that his scalp showed evidence of scaling, that he was losing his hair, not only on his head, but also on his chest and legs, and that there was some brittleness of his nails. He also complained of marked chilliness, constipation and loss of memory. In addition, the patient was on a thoroughly inadequate diet. Because of the appearance of pallor, an increase in weakness, fatigue and dyspnea, he finally consulted a physician in June, 1939. At that time he also noted vague paresthesias of the upper extremities. His local physician told him he had 'pernicious anemia' and prescribed liver, iron, yeast and various vitamin preparations. The blood picture showed an anemia, color index above 1 and a slight macrocytosis. The patient was advised as to diet and he began to consume large quantities of green vegetables in the form of carrot juice, celery and parsley. About 2 months after he started this diet, he noticed a gradual and progressive increase of yellowish discoloration in the skin, most marked on the palmar surfaces of the hands. However, despite the fact that the patient was getting adequate amounts of liver and iron, there was only slight improvement in the objective findings with the exception of some gain in weight and practically no subjective changes.

Physical examination at this time revealed a well-nourished, moderately obese male weighing 157 pounds (height 63.5 inches). There was a definite canary yellow color of the skin of the palms of the hands and the soles of the feet. There was also some slight pigmentation at the naso-labial folds. There was no evidence of skin pigmentation elsewhere. The face was puffy and sallow. Hair over the body was sparse and was absent on the thorax and legs. The finger nails were brittle. The tongue was clean and there was no evidence of glossitis or atrophy. The lungs were clear and the heart sounds were normal. Pulse rate was 62 per minute and the blood pressure was 118/80 mm Hg. The findings in the abdomen were negative, and the liver and spleen were not palpable. The genitalia were normal. Neurological examination revealed nothing abnormal; position and vibratory senses, as well as the deep reflexes, were intact.

Laboratory studies showed the following: red blood count, 3.35 M.cu.mm.; hemoglobin, 70 per cent; color index, 1.05. Urine had a specific gravity ranging from 10. 10 to 10. 20 with negative albumin and sugar and no formed elements. Electrocardiogram showed low voltage. Roentgen-ray study showed the chest was normal. In the first sample of the gastric extraction there were 86 units of free HCl and 100.6 units of total HCl. Galactose tolerance was normal. There was some increased tolerance for dextrose as shown by the glucose tolerance test. Bone marrow studies revealed no evidence of pernicious anemia or other blood dyscrasia. The basal metabolic rate was −20 per cent. The original blood lipid findings along with other determinations are given in table 1.

In view of the appearance of the patient and the low metabolic rate, the diagnosis of hypothyroidism was made.

In addition, subsidiary diagnoses were made of carotenemia and secondary anemia. Treatment was started on Feb. 13, 1941, with the administration of thyroid (whole gland) and iron. The patient's course was followed until October, 1941, at which time a final set of blood studies was made. The results of these tests, as well as those made on earlier occasions, are recorded in table 1.

Clinically, the improvement was remarkable. The sallow, puffy appearance of the face disappeared. He appeared bright and alert. There was complete disappearance of the yellowish pigmentation of the palms and soles. The nails were no longer brittle. Hair began to appear on the chest and legs. There was also an increase in the amount of scalp hair. The anemia, which had failed to respond previously to both iron and liver therapy was now controlled. There was a weight loss of 20 pounds and the basal metabolic rate was +1 per cent. Subjectively, the improvement was also striking; fatigue, weakness and dyspnea had completely disappeared and the patient had no complaints.

COMMENT

Hyperlipemia usually occurs in various forms of anemia, including pernicious anemia, hemolytic anemia and leukemia, in diabetes mellitus, nephrosis and glomerulo-nephritis, biliary disease and hypothyroidism. Laboratory tests indicated only the presence of hypothyroidism. Clinically, too, the patient represented unquestionably a case of marked hypothyroidism. The basal metabolic rate was —20 per cent, but still more important was the extremely high blood cholesterol. Concentration of this has been considered a more reliable index of the severity of hypothyroidism than the basal metabolic rate.

It is quite apparent that the patient had been ill for several years with hypothyroidism. In thyroid deficiency the pallor due to subcutaneous myxedema often is suggestive of severe anemia, which is not corroborated by the blood count. However, a moderate degree of anemia is not uncommon. Liver and iron are ineffective in the relief of the anemia unless administered with thyroid.

Carotene, or provitamin, is converted into vitamin A in the liver, where it is stored. The conversion is relatively slow and not complete; probably not more than 20 per cent of the ingested carotene is converted into the vitamin. Carotenemia is usually found in diseases exhibiting hyperlipemia, as diabetes mellitus, chronic nephritis, biliary disease and hypothyroidism. Carotene inactivates the action of thyroxin and this has been demonstrated clinically and experimentally. It then suggests the hypothesis that patients with carotenemia may exhibit a symptom complex with clinical evidence of hypothyroidism and hepatic insufficiency. In this instance, however, hypothyroid symptoms were manifested for several years before the large intake of vegetable juices. Since carotene is fat soluble and there was a marked hyperlipemia, it is highly probable that the carotene was held in solution in high concentration and could not be adequately destroyed or removed by a presumably normally functioning liver.

The administration of large doses of thyroid resulted in a marked diminution of the blood lipids and carotinoids and an increase in the metabolic rate from —20 to +1 per cent. As the result of the administration of thyroid with iron, the anemia improved considerably.

CONCLUSION

A case is presented of hypothyroidism associated with hyperlipemia, carotenemia and anemia, which responded remarkably to thyroid and iron medication.

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Synergism Between Pituitary Extracts and Chorionic Gonadotropins

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THERAPEUTIC ATTEMPTS to utilize gonadotropins for the correction of menstrual disorders have not proved to be quite satisfactory. Clinical ineffectiveness in a considerable percentage of the patients, on the one hand, and the danger of ovarian impairment, cystic or atretic changes, on the other, have not increased the popularity of gonadotropic therapy. Empirical clinical observations, however, have shown that the effectiveness of chorionic gonadotropins is augmented by simultaneous administration of pituitary extracts (1, 2). Thus it is possible to reduce the dosage of chorionic gonadotropin and minimize the undesirable alterations of the ovarian tissues.

The synergism between anterior lobe extracts and the chorionic factor was experimentally demonstrated by Evans et al. (3), Fevold et al. (4) and others. The increase in the effectiveness of the chorionic gonadotropin, according to the prevalent belief, is brought about by the pituitary gonadotropins, although the exclusive rôle of the latter in the process of synergism was questioned repeatedly. In a previous paper, we attempted to show that a pituitary extract of low gonadotropic potency is capable of producing significant weight increase and histological changes in ovaries and uteri; comparable effects were obtained also by utilizing Collip's specific metabolic principle as a synergist. A combination of minimal doses of the specific metabolic principle and of chorionic gonadotropin, each of which, when given alone was ineffective, increased ovarian weights 300 per cent and uterine weights 900 per cent.

By slightly increasing the amount of the chorionic factor and continuing its use for 10 days, even greater reactions were obtained; these equalled those described by Mazer and Ravetz (5) in their experiments on the synergistic effect of pituitary gonadotropins. Rats killed on the eleventh day showed an increase in the average for body weight from 36.5 to 70.5 gm. The ovarian weight averaged 47.5 mg. nearly 4 times that of the controls, while the uterine weight was an average of 340 mg., nearly a 23-fold increase. Using a commercial preparation which is a combination of chorionic gonadotropin and gonadotropic hormone extracted from the anterior pituitary gland[1] and injecting one 'synergy' unit, ovarian weights of 50.5 mg. were obtained, yet the weight of the uteri increased to an average of only 68.5 mg.

Microscopically, the ovaries of the animals receiving the combination of the specific metabolic principle of Collip and the chorionic gonadotropin showed transformation of numerous follicles into lutein bodies while other follicles showed moderate cystic enlargement.

EXPERIMENTAL PROCEDURE

Further studies were made to identify other pituitary factors present in the unfractionated extract which might produce synergistic effects. We used adrenotropic and thyrotropic extracts, a lactogenic preparation and a mixture of anterior pituitary factors assayed to contain 25 growth units per cc.[2] One unit of the adrenotropic factor was given as a total dose, divided into 6 injections and distributed over a period of 3 days. The animals were killed 96 hours after receiving the first injection. In a second series, chorionic gonadotropin[3] was added to the injection material for the total amount of 0.625 units per animal.

In the next experiment, thyrotropin was given for a total dose of 1.25 units, while a similar number of animals received, in addition, 0.625 units of chorionic gonadotropin.

A third group was given lactogenic hormone, 5 units as a total dose, while an equal number of animals received an added 0.625 units of chorionic gonadotropin.

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[1] This preparation (Synapoidin) was supplied by Parke, Davis & Co., Detroit, Mich.

[2] We are indebted to Dr. F. Fenger of Armour & Co., Chicago Ill., for the various pituitary extracts used in these experiments.

[3] The chorionic gonadotropin (A.P.L.) was supplied by Ayerst McKenna & Harrison, Rouses Point, New York.

The next group received the mixture of anterior pituitary factors including growth hormone in a solution containing a total dose of one growth unit per animal while the same number of animals received an added 0.625 units of chorionic gonadotropin.

In another experiment, we combined adrenotropin and lactogenic hormone, one unit of the former and 5 of the latter, alone and with added chorionic gonadotropin.

In another experiment, the same mixture of adrenotropin and lactogenic hormone was used with the addition of 1.25 units of thyrotropin. This combination was given again with and without chorionic gonadotropin.

The figures in the tables seem to show that injections of these varied pituitary factors, alone or in combination, produce a definite increase of uterine weight as an expression of their synergism with the chorionic gonadotropin. The increase in the weight of the ovary is almost negligible. The most impressive increase in uterine weight, 112.8 mg., was obtained by the combination of adrenotropin-lactogenic treatment; that with lactogen closely followed, being 107 mg. The lowest values were obtained in the series which received the thyrotropin in combination with the adrenotropin-lactogen mixture, suggesting the possibility that thyrotropin acted as a depressor upon the synergistic mechanism.

Histological examination of the ovaries revealed only a slight degree of follicular stimulation. However, one or two lutein bodies were found in each ovary, some fairly well developed, others in the incipient stage of luteinization.

The marked increase in uterine weight obtained by combining these pituitary factors with chorionic gonadotropin, seems peculiar in view of the absence of commensurate ovarian enlargement such as was noticed before with the use of the unfractionated pituitary extract or the specific metabolic principle. We must assume, nevertheless, that these immature ovaries obtained sufficient stimulation to produce the amount of estrogen and progesterone required to elicit uterine response. There is no doubt that our synergistic combinations do not affect the uteri directly, for no uterine response was obtained in castrated, immature females. We also injected some castrate rats with various dilutions of an estrone solution.[4] Six I.U. of estrone were needed to produce a uterine weight of 36.7 mg. and 25 for an increase to 57.3 mg. as compared with the uterine weight of the untreated control castrates which averaged 16.7 mg. Addition of the various pituitary factors, or of the unfractionated extract with or without chorionic gonadotropin, did not increase the uterine weights of the castrate rats treated with estrone, regardless of whether the estrone was given on the 2, 6 or 25 I.U. dosage level. These observations confirm the view that the synergistic effects take place in the ovary

TABLE 1. STUDY OF SYNERGISM BETWEEN PITUITARY EXTRACTS AND CHORIONIC GONADOTROPINS

Number of Animals	Weight Before Treatment, gm.	Weight After Treatment, gm.	Ovarian Weight, mg.	Uterine Weight, mg.	Chorionic Gonadotropin Used
Adrenotropin, 1U; 0.625 U *chorionic gonadotropin*					
7	34.0	51.6	9.4	26.0	No
7	30.0	33.4	13.0	108.0	Yes
Thyrotropin, 1.25 U; 0.625 U *chorionic gonadotropin*					
6	28.6	32.1	9.1	19.8	No
6	32.1	35.8	11.1	96.4	Yes
Lactogenic hormone, 5 U; 0.625 U *chorionic gonadotropin*					
7	33.3	46.4	11.0	25.0	No
7	29.0	33.0	14.0	132.0	Yes
Growth hormone preparation, 1 U; 0.625 U *chorionic gonadotropin*					
6	32.0	36.0	8.0	24.8	No
6	35.0	36.0	14.0	94.5	Yes
Adrenotropin, 1 U; *lactogenic hormone*, 5 U; 0.625 U *chorionic gonadotropin*					
5	36.2	32.4	7.1	22.3	No
5	35.4	33.6	13.1	135.1	Yes
Adrenotropin, 1 U; *lactogenic hormone*, 5 U; *thyrotropin*, 1.25 U; 0.625 U *chorionic gonadotropin*					
5	33.2	34.5	6.6	20.7	No
5	36.0	38.0	9.4	41.4	Yes

[4] The estrone (Theelin) was supplied by Parke, Davis & Co., Detroit, Mich.

and any changes of the uteri are the results of the production of ovarian hormones. It seems, however, that ovarian size is not an absolute criterion of ovarian secretory activity since it is possible to obtain substantial uterine responses without far-reaching changes of ovarian structure.

The presence of pituitary gonadotropin in the various extracts used must be considered as a possible explanation of the effects observed. However, the marked augmentation of effects with the small amounts of chorionic gonadotropin would make it necessary to postulate a concentration of gonadotropins to a degree which is incompatible with the complete ineffectiveness of these extracts if used alone. The assumption, moreover, that the thyrotropic extract owed its synergistic potency to the presence of gonadotropin in significant amounts, is contradicted by the fact that its addition to the lactogenic-adrenotropin-chorionic gonadotropin mixture elicited a drop of uterine weight from 135 to 41 mg., instead of further augmentation.

SUMMARY

Synergistic effects, similar to those obtained by the combination of unfractionated anterior lobe extracts or Collip's 'specific metabolic principle' with chorionic gonadotropin, are demonstrable if purified pituitary fractions are combined with chorionic gonadotropin. Adrenotropin, thyrotropin, lactogenic hormone, 'growth extracts' and combinations of adrenotropin and lactogen, as well as adrenotropin, lactogenic hormone and thyrotropin were used. Increase of uterine weight, comparable to that previously reported, was observed although the ovaries did not show significant enlargement.

As the synergistic combination is ineffective in the castrate, in spite of administration of estrogen, it must be assumed that the effects are due to ovarian stimulation and production of ovarian hormones. Microscopic examination of the ovaries revealed slight follicular stimulation accompanied by localized luteinization.

Uterine response obtained with a minimum of ovarian changes appears to be a more physiologic reaction than uterine enlargement of the same or lesser degree preceded by extensive ovarian changes.

These findings are in accord with clinical experience which suggest that better therapeutic results are obtained if a lower dosage of the chorionic gonadotropin is used in combination with synergistically effective anterior lobe extracts.

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COMMUNICATIONS TO THE EDITORS

Use of Ethinyl Estradiol to Prevent Lactation in Puerperal Patients

ETHINYL ESTRADIOL was prepared by Inhoffen and Hohlweg[1] by the replacement of the 17th carbon atom in estradiol by an ethinyl group. When given subcutaneously in oil, ethinyl estradiol and estradiol are equal in potency, but when given orally, ethinyl estradiol is 15 to 20 times as active as estradiol. Clauberg and Ustun[2] reported the use of ethinyl estradiol in secondary amenorrhea and oligomenorrhea and demonstrated proliferative changes in the uterine mucosa following doses of 56 mg. of ethinyl estradiol, given over a period of 20 days. No symptoms of toxicity were reported in their series. Salmon et al.[3] administered ethinyl estradiol in the form of enteric-coated tablets and in alcoholic solution to 27 patients with menopausal symptoms and morphologic evidence of estrogen deficiency (tablets, 22 patients; alcoholic solution, 5 patients). Ethinyl estradiol in the form of enteric-coated tablets was found to possess high estrogenic potency when administered orally, as demonstrated by the relief of menopausal symptoms and by the characteristic estrogenic changes induced in the vaginal mucosa. Nausea and vomiting occurred in 4 of the 22 patients taking the enteric-coated tablets and in all 5 patients taking the alcoholic solution of the hormone. The side-effects included abdominal pain and malaise, chills and vertigo.

We are reporting a series of 59 cases in which ethinyl estradiol was administered orally to puerperal women, in order to prevent lactation. These patients had no other form of therapy; no saline cathartics or limitation of fluids were employed. Breast support in the form of a brassiere was used in some cases. These cases can be divided into 3 groups, as follows.

Group 1. The amount of ethinyl estradiol administered to 26 women was between 0.45 mg. to 0.90 mg. daily for 2 to 3 days. Medication was started 1 to 2 days post partum, except in 4 cases, in which treatment was started on the 3rd, 4th, 6th and 56th day post partum, respectively. Of this group, 9 women had fullness of the breasts ranging from moderate to full engorgement, with lactation the general rule. In some of the patients, the engorgement did not begin until the 8th or 9th day, and even as late as the 14th day post partum. Poor results were obtained in two women in whom dead fetuses were carried 1 and 3 days ante partum, although treatment was not begun until after delivery. Results were good in 17 patients. In 12 women there was no secretion from the breasts whatever; however, some of these patients may have lactated after going home on their 10th day post partum. This group was composed entirely of service patients, and followup study was difficult. Weaning of the baby at 8 weeks was entirely successful and satisfactory, the breast secretion disappearing within 72 hours; the dosage given was 0.90 mg. daily for 2 days.

Group 2. The dosage given was between 1.35 and 2.40 mg. of ethinyl estradiol daily for 3 days, beginning 1 to 2 days post partum. There were 16 patients in this group, and the results were excellent in 12 women. In 4 cases, there was some fullness of the breasts, but only in one case was there any marked engorgement or distress. This patient had received 1.80 mg. daily for 3 days beginning one day post partum.

Group 3. The dosage of ethinyl estradiol was 1.80 to 2.40 mg. given daily for 3 days, and started 4 to 21 days post partum. These women had, in most instances, already begun to nurse their babies, and breast feeding was discontinued for various reasons. There were no failures of therapy in this group. The breasts, if previously full and engorged, subsided within 24 to 36 hours. However, when there had been lactation already, there was continuance of milky secretion as a rule for 4 to 14 days. One patient continued to secrete for a month although there was no associated breast fullness or pain.

Discussion. There seems no question that with administration of sufficient ethinyl estradiol, lactation either will be prevented entirely or controlled so well that the large majority of patients avoid the usual aftermath of symptoms, such as painful, engorged breasts, fever and discomfort. Some patients respond better than others; however, when the dosage given is between 1.80 and 2.40 mg. daily for 3 days, good results can be expected in at least 90 per cent of the cases.

There were no toxic symptoms exhibited in any of these 59 patients, even though therapy was begun in one instance 3 weeks, and in another 8 weeks after delivery of the child. The drug was tolerated well, with no abdominal distress, nausea, vomiting or weakness.

Summary. A series of 59 women, all puerperal, were given ethinyl estradiol orally, for 3 to 4 days.

When the amount given was below 1.5 mg. per day, results were only fair, with 9 failures in a series of 26 cases.

When 1.5 to 2.4 mg. of ethinyl estradiol was given daily for 3 days, and the medication was started 1 to 2 days post partum, the results were excellent in 12 cases. There was only one frank failure.

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When the dosage was 1.80 to 2.40 mg. daily for 3 to 4 days, given 4 to 21 days post partum, relief was quickly obtained, and the results very satisfactory.

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EDITORIALS

CHONDRO-EPIPHYSITIS AS A MANIFESTATION OF THYROID DEFICIENCY

THAT THE THYROID hormone has a stimulating effect on cells throughout the body has long been a medical truism. A necessary corollary to that generalization, however, has often been overlooked. Deficiency of thyroid secretion must necessarily affect the chemical processes, hence the vitality, of all organs and tissues. If no particular organ or system is particularly vulnerable, the result is likely to be a condition of 'general poor health' to use McLester's characterization. When, however, some one part of system is, for genetic or other reason, of less than proportionate intrinsic vigor it is likely to be more affected than other parts of the body. In the older terminology it becomes a *locus minoris resistentiae*. This principle seems to account for the fact that thyroid deficiency may be manifested in numerous sorts of symptomatology—sorts that seem, at first thought to be too dissimilar to have resulted from a common etiologic factor.

As was brought out strikingly in a symposium on the relation of the endocrines to skeletal development at the recent Atlantic City meeting of the Association the epiphyseal structures are exquisitely responsive to hormonal influences. The influence of the thyroid hormone is emphasized in a recent article by Schaefer and Purcell.[1] These authors, following recent usage, bring together under the term 'juvenile chondro-epiphysitis' a number of disorders which have appeared under a variety of names such as Legg-Perthes disease, Osgood Schlatter disease and Schevermann disease; the specific term referring to the site of manifestation. They do not, however, indicate any essential difference in the fundamental nature of the disorders. The etiology has long been uncertain. Trauma, infection, toxemia, heredity or other factors have been postulated as causes but without convincing evidence of the fundamental etiological importance of any.

The authors report a detailed study of 27 cases of chondro-epiphysitis in which remarkable improvement was seen when thyroid, in adequate dosage, was administered. As accessory measures, ambulatory general management and reduction of obesity were emphasized. Anterior pituitary preparations were also given in some cases when those were regarded as specifically indicated. The patients ranged in age from 5 to 16 years and the dosage from 2 to 3.5 grains of desiccated thyroid daily. The period of treatment extended in various cases from 2 months to 4 years.

The current methods of immobilization and orthopedic treatment are condemned. The conviction is expressed that the proper basic treatment is adequate thyroid therapy. In consonance with this conclusion the authors propose discarding the confusing terminology of proper names as related to the various joints involved and suggest the term 'osteochondreal hypothyroidism' as more descriptive and as accurately applying to all of the varieties.

It should be added that the authors confine their discussion to juvenile osteochondreal hypothyroidism, excluding such adult manifestations as Kienbock's disease and osteo-chondritis dissecans.

R.G.H.

[1] SCHAEFER, R. L., AND F. H. PURCELL: *Am. J. Surg.* 54: 589. 1941.

AMINO-ACID FACTOR IN MALE FECUNDITY

FOR MANY YEARS clinicians have recognized that obesity is likely to be associated with low fecundity. Since the subjects appear to be over-nourished, the fact that they frequently suffer from protein food deficiency is often overlooked. Indeed, the very obesity itself is sometimes, in part at least, to be ascribed to the lack of the specific dynamic action of protein metabolites. Poultrymen and stock breeders have long known that some protein foods are more valuable than others in promoting both vigor and fecundity. The part played by the individual amino-acids in determining the biological value of proteins as related to fecundity has recently come under new study.

At the 1942 meeting of the Federation of American Societies for Experimental Biology, L. Emmett Holt and collaborators of the Department of Pediatrics, Johns Hopkins University Medical School, reported an investigation of some of the effects of amino-acid deficiency in man. Four men and one woman volunteered to serve as subjects over periods in which different amino-acid deficiencies were established. The lack of both tryptophan and lysine were found to result in negative nitrogen balance which persisted throughout the experimental period, except that the female subject was able to maintain the balance for a brief period preceding the onset of menstruation. When 3 of the subjects were given arginine for 10 days, they were able to maintain nitrogen equilibrium but a prompt deleterious influence upon the gonads occurred. On the 9th day of the experiment the spermatozoal count had been reduced to 0.1 of the normal value. On a similar diet, but containing arginine, the sperm cell count began to improve at once. Several weeks on a normal diet, however, were required for complete restoration.

R.G.H.

Abstracts of

CURRENT CLINICAL LITERATURE

Editor: DANIEL A. MCGINTY. *Collaborators:* E. B. ASTWOOD, ISRAEL BRAM, JOHN C. BURCH, JOHN C. DONALDSON, MURRAY B. GORDON, E. C. HAMBLEN, FRANK A. HARTMAN, R. G. HOSKINS, J. E. HOWARD, J. P. PRATT, J. T. LEWIS, JOSEPH M. LOONEY, A. E. MEYER, C. A. PFEIFFER, BORIS B. RUBENSTEIN, EMMERICH VON HAAM.

ENDOCRINE GENERAL

BENNETT, H. G., AND R. W. TE LINDE

The menopausal syndrome. Treatment with the implantation of crystalline estrone pellets.

Satisfactory relief of symptoms with theelin pellets occurred in 93% of the patients treated while diethylstilbestrol pellets gave 100% of relief. Abnormal bleeding occurred in 3.5% of the theelin treated patients while 72% of the diethylstilbestrol patients had abnormal bleeding. Of the seven forms of therapy tried the implantation of crystalline estrone pellets was the best.—*C.P.*

BRONSTEIN, I. P., S. WEXLER, A. W. BROWN AND L. J. HALPERN.

Obesity in childhood. Psychological studies. *Am. J. Dis. Child.*, 63: 238, 1942.

Comprehensive physical studies of 35 obese children did not reveal any ascertainable evidence of any endocrine basis for the condition. The mean intelligence of this group was above the mean of the population as a whole and achievement tests showed little or no difference from children of similar capacity. Only 2 of the group showed a tendency toward feminity. There was a definite tendency to be sensitive about their obesity, toward extroversion and instability and an interest in sedentary play activities. —*M.B.G.*

BUNKLEY, T. F.

Uterine bleeding complicating pregnancy. Its significance as shown in one hundred consecutive cases. *Texas State J. Med.* 37: 672. 1942.

In 100 consecutive cases of uterine bleeding occurring during pregnancy, 63 cases occurred during the first trimester, 22 during the second, and 19 during the third.

Thirty-two cases of hemorrhage occurred with no pain at any time. During the first two trimesters there were 51 cases of threatened or inevitable abortion and 25 cases of ectopic pregnancy. The other cases of bleeding occurred chiefly during the last four months, representing 15 definite abnormal conditions, including 8 cases of placenta praevia and 4 abruptio placentae. Of the 19 cases of bleeding occurring during the last trimester, there were 14 live babies. In this series there were 3 maternal deaths, 2 in the last trimester as result of uterine atony, and 1 during the first trimester due to ectopic pregnancy complicated by chronic nephritis.

The conclusion drawn indicates that bleeding during pregnancy in any stage is a symptom of trouble of some kind and in the majority of cases is serious either to the fetus or the mother. During the third trimester bleeding of any severe type may be fatal to both the mother and the baby.—*Author's summary.*

FINCH, J. WILLIAM.

The nausea and vomiting following administration of diethylstilbestrol. *Jour. A.M.A.* 119: 400. 1942.

The naeusea and emesis accompanying diethylstilbestrol therapy is similar to if not identical with that of early pregnancy. The author believes this is an allergic response and desensitization with small but gradually increasing doses results eventually in the full therapeutic dose being well tolerated.—*C.P.*

HAIN, A. M.

Further observations on the role of progesterone (pregnanediol) and oestrogen in pregnancy. *J. Endocrinol.* 3: 10. 1942.

The progesterone excretion (pregnanediol) of over 100 women with histories of previous miscarriages or anomalies was ascertained throughout a pregnancy in which some form of therapy was usually administered. The common theory that parturition and abortion are due to the waning secretion of progesterone was found untenable, as both conditions were frequently accompanied by levels of pregnanediol output associated with a continuance of pregnancy. Also in over 60% of women in whom symptoms of threatened abortion were present the outputs of both gonadotropin and pregnanediol were such as characterize normal pregnancy. However, pregnancy was capable of being maintained by even small amounts of progesterone as was shown by the degree of fetal development reached by patients in whom abortion occurred at low levels of pregnanediol excretion. It is concluded that a factor making for abortion (or parturition) comes into play which progesterone is powerless to inhibit. The possibility that this was a rise in free estrogen was investigated but not substantiated. Other possibilities are discussed and evidence is given of an extraneous factor exercising a rhythmic control of hormone excretion at the approach of parturition. The data afforded information as to secretory types and the prognostic value of the analyses, and permitted a comparison to be made of the values of vitamin E and progesterone therapy in threatened and habitual abortion; the importance of rest was stressed.—*Author's summary.*

JOHNSTON, MARGARET W., AND L. H. NEWBURGH.

Calculation of heat production from insensible loss of weight. *J. Clin. Investigation* 21: 357. 1942.

Indirect calorimetry i.e. the calculation of heat production by the measurement of vaporization provides satisfactory values provided that suitable factors for the translation of weight into heat are employed. These factors depend upon the type of diet fed: 57 for the low carbohydrate diet, 50 for the high carbohydrate diet and 53 for the normal or ordinary diet. These factors multiplied by the hourly insensible weight loss will give a satisfactory prediction of the 24 hour heat production.—*B.B.R.*

KALTREIDER, N. L., G. R. MENEELY, AND J. R. ALLEN.

The effect of epinephrine on the volume of the blood. *J. Clin. Investigation* 21: 339. 1942.

Plasma volume was determined from the disappearance slope of injected azo blue T. 1824 in six normal subjects, two patients with polycythemia vera, and in two splenectomized patients. In the normal individuals following the injection of 1 cc. of epinephrine 1–1000 the plasma volume decreased approximately 5%, and the red cell count increased about 2%. The pulse rate and systolic blood pressure was increased while the diastolic blood pressure diminished. Similar findings occurred also in the patients with polycythemia vera, while after splenectomy the decrease in plasma volume after epinephrine is accompanied by a diminution in red cell count. The injection of epinephrine in normal individuals thus resembles the effects of severe exercise.—*B.B.R.*

McLAREN, H. C.

The normal menopause. *J. Obst. & Gynaec. Brit. Emp.* 48: 1. 1941.

A study of 84 patients (average age 63) who had undergone a normal menopause refuted the generally accepted statement that atrophy of the genital tract with thinning of the vaginal mucosa is the usual sequel to the climacteric. Histological examination of the vagina revealed that 65% were normal in appearance. Grade III smears (predominantly composed of squamous cells) were found in 78%. A pure growth of Döderlein's bacillus was present in 28%, and in 8% there was secretion with a PH of less than 5. Evidently in many females little anatomical or physiological change occurs in the post-menopausal vagina, except for an occasional rise in PH with an influx of mixed organisms. The findings provided confirmatory evidence of ovarian activity after the menopause.—*H.O.H.*

McLAREN, H. C.

The induced menopause. *J. Obst. & Gynaec. Brit. Emp.* 48: 23. 1941.

Examination of 214 cases of induced menopause (100 radium treated, at least 2400 mg.-hours, 39 hysterectomy-oophorectomy, 36 hysterectomy-unilateral oophorectomy, 39 hysterectomy) revealed that flushing occurred in about ½ of the radium and castrated groups, but was rare in patients following hysterectomy with preservation of ovarian tissue. No marked changes occurred in the external genitalia. Mucosal atrophy of the vagina occurred in 14% of the radium group, 28% of the castrated group, 3% of the surgical group with retained ovarian tissue. Upper vaginal stenosis was common in the radium group. No relation existed between the severity of menopausal symptoms and the type of vaginal smear or mucosa. The use of the vaginal smear as an index of the success of estrin therapy proved indefensible.—*H.O.H.*

MOEHLIG, R. C., AND L. JAFFE.

Syndrome simulating diabetes insipidus in dogs induced by desoxycorticosterone acetate. *J. Lab. & Clin. Med.* 27: 1009. 1942.

Two of five dogs receiving 5–10 mg. of desoxycorticosterone acetate daily over a period of four months developed a syndrome of polydipsia and polyuria, and one of them died. Muscular weakness and tetany were prominent features in all five dogs. Microscopic sections of the hypothalmus showed localized encephalitis. A patient suffering from myasthenia gravis received multiple pellet implantation totaling 1300 mg. of doca. Within two weeks he too developed marked polydipsia and polyuria and spontaneous tetany. The symptoms diminished in severity over a three month period, since when the patient has felt stronger than at any time since the onset of his illness. Thus tetany, polydipsia, and polyuria may be considered toxic manifestations of over-dosage with doca.—*B.B.R.*

MUKHERJEE, C.

The posterior pituitary factor in toxaemias of pregnancy. *J. Obst. & Gynaec. Brit. Emp.* 48: 586. 1941.

Experimental observations, using blood ultrafiltrates of 50 unselected cases of toxemias of pregnancy, revealed a substance which showed melanophoric, antidiuretic, and vasopressor properties, as tested in the frog, the guinea-pig, and the cat, respectively. The reaction of the toxemic subject to injection of vasopressin was greater and more sustained than that of the normal pregnant female. Toxic symptoms in normal pregnancy required massive doses of vasopressin, whereas a single small dose (¼ cc.) produced marked toxemic manifestations under conditions of pregnancy toxemia. These facts, together with the observation that in the eclamptic subject injections of vasopressin elicited a blood pressure rise quite out of proportion to size of dose, led to the concept of a high concentration of the pressor hormone in the circulation, with perhaps a hypersusceptibility on the part of the patient. A functional hyperactivity of the posterior lobe in toxemic states is postulated.—*H.O.H.*

SAPHIR, W., AND A. R. WEINGLASS.

Severe angioneurotic edema following diethylstilbestrol therapy. *J. Am. Med. Assoc.* 119: 557. 1942.

Diethylstilbestrol at a dosage level of 0.5 mgm. daily for 6 days produced severe allergic edema in a 53 year old menopausal female. After mild edema of the hand and foot, severe edema of the lips, face, neck and tongue appeared.

Fearing edema of the glottis the patient was hospitalized but the symptoms subsided in 24 hours. Intradermal tests with 0.1 cc. of a 1:10,000 solution were positive.—*C.P.*

STERNBERG, W. H., AND V. JOSEPH.

Osteodystrophia fibrosa combined with precocious puberty and exophthalmic goiter. Pathological report of a case. *Am. J. Dis. Child.* 63: 748. 1942.

About 26 probable cases have been reported of the syndrome characterized by asymmetric diseases of bones, melanotic pigmentation and precocious puberty in females. A similar syndrome without precocious puberty occurs less frequently in males. The authors report what they consider to be the first complete pathological observations of this syndrome. The clinical aspects of the case were reported by McCune and Bruch (*Am. J. Dis. Child.* 54: 806. 1937).

The disease of the bones is patchy and is characterized by lacunar osteoclastic resorption, fibrosis of marrow spaces, osteoblastic repair and cyst formation. The presence of islands of cartilage with bone formation at their peripheries is looked on as an extreme effort at repair. The pathologic picture resembles von Recklinghausen's disease although the tempo of bone resorption is slower and hyperparathyroidism is not present.

The important endocrine findings were a hyperplastic thyroid, thymus and lymphoid structure, mature cystic ovaries with no evidence of luteinization, a narrow, lean adrenal cortex, and parathyroids within normal limits. The pituitary showed basophilic hyperplasia with adenoma formation.—*M.B.G.*

SULLIVAN, J. M., AND R. A. MUNSLOW.

Gynecomastia. A study of five cases. *J. Am. Med. Assoc.* 118: 1443. 1942.

Five cases of gynecomastia in soldiers are presented in which the chief complaint was inability to wear a pack. While the onset of the swelling was during adolescence, no accompanying endocrine symptoms were present. The pathological changes were histologically similar to those of chronic cystic mastitis.—*C.P.*

VIETS, H. R., R. S. SCHWAB AND M. A. B. BRAZIER.

The effect of pregnancy on the course of myasthenia gravis. *J. Am. Med. Assoc.* 119: 236. 1942.

After reviewing equivocal data from approximately 20 cases in the literature, the author adds the case histories of eight myasthenic patients who have undergone pregnancy and concludes that the effect of pregnancy on myasthenia is usually favorable. Exacerbation if it occurs is usually in the first trimester while amelioration occurs in the second and third trimester and extends for 3 to 6 months after delivery.—*C.P.*

ZONDEK, BERNHARD.

Simplified hormonal treatment of amenorrhea. *J. Am. Med. Assoc.* 118: 705. 1942.

The simplified treatment of amenorrhea for the general practitioner is outlined as follows:

1. Secondary amenorrhea of 2 years duration—50 mg. progesterone over two to five days.

2. Less than two years duration—25 mg. progesterone with 2.5 to 5.0 mg. estradiol benzoate over a period of two days.

3. Primary and castration amenorrhea—50 mg. progesterone with 2.5 to 5.0 mg. of estradiol benzoate in 5 days.—*C.P.*

PANCREAS

BARNS, H. H. F.

Diabetes mellitus and pregnancy. *J. Obst. & Gynaec. Brit. Emp.* 48: 707. 1941.

A review of 25 diabetic pregnancies in 21 patients revealed the average age at onset of diabetes to be 31 years; the average age at which pregnancy supervened to be 33 years. Insulin requirements increased in 74% of cases as pregnancy progressed. The maternal prognosis was good and pregnancy did not aggravate the diabetes if the latter was adequately handled. Fetal mortality was 44%. Toxemia of late pregnancy was held the chief cause of intra-uterine death, twice as lethal to the fetus of the diabetic subject as to that of the non-diabetic female. Hypoglycemia was not found in any of the live infants. However, administration of extra sugar in early neonatal life is advised to counteract any possibility of this complication. Management of the pregnant diabetic is outlined.—*H.O.H.*

LUETSCHER, J. A. JR.

Metabolism of amino acid in diabetes mellitus. *J. Clin. Investigation* 21: 275. 1942.

In a group of twelve severe untreated diabetics high plasma levels of amino acids accompanied by increased urinary excretion of amino acids were found. Insulin therapy reduced the blood and urinary excretion to normal levels which were maintained despite fluctuations in the blood sugar and urinary sugar excretion.—*B.B.R.*